

Is there a future for Russian science?

If the fighting on the streets of Moscow gets out of hand, Russian science will be finished, but this or the next Russian government needs outside advice on how it should reorganize the research enterprise.

WHAT future is there for Russian science? A few weeks ago (see *Nature* 365, 283; 23 September 1993), President Boris Yeltsin asked for opinions on this important question from the Ministry of Research and from the Russian Academy of Sciences. He then ensured that it would not be answered quickly by telling the Russian Parliament (the Congress of Deputies) to pack its bags, go home and await the outcome of a general election. Yeltsin (and the rest of us) should be glad that he does not already have a civil war on his hands; what the days and weeks ahead will bring is another matter. If the street fighting turns into a second civil war, or if the Russian Federation instead disintegrates into some of its component parts, the future of Russian science will be an academic question. Otherwise, the question will have to be answered. What follows is a simple recipe either Yeltsin or his successor could take up.

The starting point must necessarily be the Russian Academy of Sciences, and the view that it is too important to be dispensed with. Founded by Peter the Great, and shaped into a free-standing instrument of scholarship with the help of the German mathematician Euler, it is and should remain the custodian of the conscience of basic science in Russia. But the academy has grown since 1917 to be much more than that — and much less. Even now, it is not fully appreciated how far it has been corrupted, first by Lenin's concept of science as an instrument of revolution and then by Stalin's practice of seducing academicians away from principled care for the condition of Russian science by offering them personal power and privilege in return for their connivance in unjust illiberality.

Price

Why else is the academy still struggling to keep its hold on the discredited trappings of past power? Three years ago, when Yeltsin, as president of the Russian Federation, cut the ground from beneath Mikhail Gorbachev (then president of the Soviet Union) by inventing the Commonwealth of Independent States, the old Soviet academy deftly hijacked the then embryo Russian academy, taking the role for itself. Part of the price it had to pay was the appointment by the Russian government of a new president, Yuri Osipov, a mathematician and an old friend of Yeltsin from Sverdlovsk, who has not been a great success. What seemed for a time to be the prize was the right to manage the old network of research institutes and the budget that went with that. To its shame, but not, apparently, its embarrassment, the academy's bureauc-

racy has occupied a building in Moscow whose chief distinction is the inappropriate opulence of its vulgarity.

This is entirely out of character with what should be a scholarly academy's way of doing business. But further self-denial is required. It is also essential that the academy should have no part to play in the management of the research establishments grown huge in Stalin's time in the belief that they would spearhead both industrial and military innovation. At last, under the benign influence of Mr Boris Saltykov, the research minister, the Russian government seems to have grasped that truth. The academy has not yet come to terms with it. That is why the academy has become part of the problem, not the solution.

Goals

The academy should thus set itself three goals (or have them imposed upon it). First, it needs to earn the name 'academy' by seeing that its members are elected (exclusively by existing members, not on instruction from ministers) on merit alone. Second, it should agree to administer on the government's behalf those scientific institutions whose disappearance would be a loss to basic science and to Russian science, but for which there is no other home. Third, it should offer itself as a source of impartial and informed advice to the Russian government on technical issues within its competence. To make this change of direction credible, it should fight shy of the administrative tasks from which its power of patronage has traditionally derived. It should move to more modest quarters, perhaps most easily by moving back to St Petersburg (where it began). That stark recipe would not be the death sentence many members of the academy will think it. Rather, it is the only realistic route to survival.

Taking the major research institutes away from the academy does not mean that they should be abandoned. Many of them are too important, and many of their people are too talented, for that to be contemplated seriously. As an interim expedient, direct support by the research ministry would be the best course. Then the government could decide what should be done with them and with the hundreds of other research institutes it has inherited from other academies (for medical and agricultural sciences) as well as from the technical ministries and the military enterprise. There are two immediate needs. The first is to staunch the flow of the most talented people from research, either to jobs abroad or to jobs in Russia that have nothing to do with science. The second is to find a seemly way of dispensing with the services



of those who clog the institute payrolls, but who have long since ceased to be productive as researchers.

But how to decide which institutes are worth keeping, and which people have something to contribute in the years ahead? In the turbulent few years past, the Russian research enterprise has been partly shaped by what might be called market forces; research institutes able to recruit funds from outside their traditional sources of support have been able to pay their people decent salaries; others have been compelled to manage on a shoe-string. But that, even if it works, is a blunt instrument. Laboratories now prospering may owe more to the guile of their directors, or to lucky accidents, than to their potential value to Russia. A further difficulty is that the present organization of research is ill-suited to modern needs. Basic research in, say, genetics remains largely the prerogative of institutes of the academy, but clinical applications rest with the Academy of Medical Sciences. The gulf between research and the universities is a Stalinist scandal.

But Yeltsin seemed, a few weeks ago, to be hoping that competition for research grants would allow an extension of that process, allowing competent researchers to win continuing support and forcing others out. There are several snags. One is that the criteria by which research-grant applications should be judged must somehow be defined. Another is that research cannot happen in a vacuum, but requires infrastructure. A third is that the Russian research community has no experience of making honest appraisals of its own research proposals. The experience of the Soros fund, which plans to allocate \$60 million to basic research proposals in the next few months, will be enlightening, but cannot be a model for all of Russia's need.

Yeltsin (if he survives the present turmoil) should therefore bite an unpalatable bullet, and seek help in answering his question from outside Russia, both to shrink the research enterprise and to reshape it. Russian science has well-wishers enough. It should not be difficult to find a group of outside commissioners to recommend ways of shrinking and reorganizing the plethora of research institutes and then of encouraging the remaining productive core. It would be a huge task, but not an impossible one. And there are precedents. The Baltic states have sought help of just this kind from their Scandinavian neighbours. For that matter, research organizations in Germany and (now) in Japan as well seem to value external advice on their way of doing business. Why should not even Russia follow suit?

Pride is one answer. Another is that an external assessment of Russian science would be denounced by some of those adversely affected as a malign plot by, say, the US Central Intelligence Agency or some other imagined Western instrument of Russia's emasculation. The second is a powerful political argument. But it is irrelevant to the question of how to salvage something worthwhile from one of the largest and most talented components of the international research enterprise. Every political upheaval in the past few turbulent years has taken its toll of Russian science. The storming of the Russian parliament on Monday, whatever the justification, will be another setback. How many more of these crises will be needed to kill it off? □

India's latest earthquake

The seismic causes of last week's tragedy are less forbidding than the ultimate cause: rural poverty.

THE real tragedy of last week's earthquake in Maharashtra State is that an event of such a magnitude (6.4) in California would not have killed 20,000 people (but the final toll in India may be even higher). Quite possibly, there would have been no deaths at all, while damage to houses and other buildings would probably have left only a small mark on the financial accounts of US insurance companies. That was the experience in 1987 of the earthquake (magnitude 6.0) near San Jose, when there were no deaths and the physical damage amounted to some \$10 million. Earthquakes as such are not especially damaging to people, who are maimed and killed by the damage done to buildings and other mechanical structures. Reports that farm animals quartered overnight in nearby fields have survived bear that out.

It is, of course, cruel bad luck that last week's earthquake should have happened in a region in which earthquakes are rare and, far away from the edges of the neighbouring tectonic plates, are expected to be rare. Most of central India, indeed, lies on Precambrian continental crust, which experience (as in the Canadian Shield) has shown to be remarkably stable against seismicity. But the Indian Plate is still being impelled northwards, into and beneath the Himalayas. The seismic trace of last week's earthquake (with a focus 20 km below the surface) accords with the view that the cause was a thrust fault caused by north-south compression in the plate. That is no comfort, of course, to the survivors.

Nor will it be much help to them that the Indian government's political opponents in a forthcoming state election have seized on local anxiety about a string of small earthquakes in recent years to claim that the government should have taken action sooner. It might be different if there were a better understanding of the significance of the microseisms that sometimes precede destructive earthquakes. But in any case, what could the government have done?

The reasons why so many were killed in Maharashtra last week have much longer roots than the period in which the local population has been worried by microseisms. The standard way of building houses in the region, in which rocks are embedded in adobe, is a recipe for personal disaster when earthquakes come. So why should not Maharashtra follow California in building wooden frame houses to prescribed building standards? Because India does not have all that much wood, because earthquakes are in any case uncommon in the region and because the people seeking shelter in central Maharashtra could not afford the luxury of safe housing. In short, if the proximate cause of last week's tragedy was the earthquake, its underlying cause was the continuing poverty of rural India. The question to ask is not whether the microseisms of the past few years could have been used to avoid the tragedy, but when India's new-found industrial prosperity will trickle down to relieve some of the most cruel poverty in the world. □

MRC research centre under threat from London health service plans

London. A question mark has been placed over the future of a multimillion pound flagship biomedical research centre being built by the Medical Research Council (MRC) and the Wellcome Trust by uncertainty over the reorganization of London's teaching hospitals.

The MRC has already spent £13 million on the construction of a five-storey building to house its new Clinical Sciences Centre (CSC) at the Royal Postgraduate Medical School (RPMS), at Hammersmith Hospital.

The Wellcome Trust has agreed to invest an additional £4 million to pay for the construction of one floor of the building, due for completion in March 1994. Wellcome has also promised to provide programme grants to five scientific teams.

But the government is now proposing to merge the clinical services of the Hammersmith Hospital with those of the Charing Cross Hospital. The merger will form part of a larger streamlining of clinical services in London as part of the introduction of a full internal market in the National Health Service.

If the decision is made to close Hammersmith, its research laboratories would have to move to the site of Charing Cross Hospital, about three miles away. Such a move would not take place for several years. But hospital officials are worried that it would

create a 'blight-and-flight' syndrome as grant-awarding bodies become reluctant to commit further funds to the centre, and scientific staff seek more secure positions elsewhere.

The MRC is planning to use the Hammersmith site to re-house laboratories from its Clinical Research Centre (CRC) in north London, which is due to close by the end of 1994. Tenured contracts have already been awarded to a number of CRC team leaders and other leading biomedical research workers from Britain and abroad.

Management consultants KPMG Peat Marwick have been assessing both hospital sites. They are due to report to a steering committee representing the London Implementation Group, the body set up by the government to advise on future plans for London's hospitals, by 20 October. This group is itself due to make recommendations to the Health Minister, Mrs Virginia Bottomley, by the end of the year.

According to figures presented to the steering committee's project group last Friday (1 October), the cost of transferring research activities from the Hammersmith to Charing Cross Hospital sites, which are already cramped for space, would be about £105 million. In contrast, moving clinical services from Charing Cross to Hammersmith Hospital would cost £55 million.



The new centre is to open in 1994, but for how long?

Neil Gershon, secretary of the RPMS, says that Hammersmith offers more than just financial advantages. In particular, it has room to expand into playing-fields behind the hospital, and is already engaged in a new phase of building and investment in modern clinical facilities.

Political factors, however, could favour the Charing Cross Hospital site, which was opened in 1972. It is easier to reach by public transport than the Hammersmith site and falls within a political constituency with a slim conservative majority. Closure of the Hammersmith site is also thought to be favoured by the North West Thames Regional Health Authority.

Kay Davies, professor of genetics at the Institute of Molecular Biology in Oxford, who has been appointed director of the CSC, remains optimistic about the future of the centre. Even if the Hammersmith is relocated to Charing Cross, she says, the need to obtain planning permission and construct new facilities will delay any move until after the CSC is well-established. "Even if the worst scenario happened and we had to move in five or six years time we would already be flying".

But David Evered of the MRC admits that a second move would be highly disruptive for research teams. "If the outcome was such as to require a relocation, that would inevitably cause perturbation in the system," he says. If the move does take place, he says, it would be "an absolute prerequisite that certain conditions were met".

The Wellcome Trust had been expected to make final decisions on a number of matters, including the appointment of research teams to occupy the fourth floor, in September. But it is not now planning to make final decisions on appointments until November.

Davies says that Wellcome is right to be cautious. "I'm sure they won't want to fund things on this site unless they believe it's safe", she says. The trust itself has declined to comment.

Julie Clayton

NSF funds switched to facilities

Washington. The National Science Foundation, the main government agency for the funding of engineering and the physical sciences in US universities, will receive a seven per cent increase in its budget in the financial year beginning this month. The total will rise from \$1.86 billion last year to \$1.99 billion.

Final figures agreed by Congress last week will trim \$220 million from the research funding requested for the NSF by the Clinton administration. This will allow the budget for new facilities and instruments to double in the coming year, from \$50 million to \$100 million.

The increase for buildings and equipment was agreed in a conference between House and Senate representatives on the section of the administration's budget bill which funds NSF and NASA. It reflects a growing feeling in Congress that agencies such as NSF should be given adequate funds for new research facilities to avoid universities having to rely on "earmarked" projects pushed through by friendly congressmen. The NSF will invite proposals from univer-

sities this winter for projects on which to spend the new money.

A proposal by the Senate side that the NSF's request for High Performance Computing and Communications (HPCC) should be cut back by \$50 million was scaled down in conference. As a result, HPCC funding will now grow sharply to \$305 million, against \$225 million last year.

Language in the Senate version of the bill, which criticizes the NSF for its emphasis on pure research and calls for 60 per cent of its funds to be spent on so-called "strategic research," will remain in place. However, the language is not binding on the foundation.

The appropriations bill, one of thirteen disparate segments of the entire US budget now passing through Congress, allows the National Aeronautics and Space Administration (NASA) to spend \$2.1 on its planned space station next year. But it confirms the demise of SETI, NASA's small but controversial programme to seek out extraterrestrial life (see *Nature* 365, 380; 1993).

Colin Macilwain

Conservationists fear defeat on revised flood control policies

St Louis, Missouri. "How come we lose so many ball games when we're all so sincere?" This question, first posed by cartoon character Charlie Brown, is already being asked by conservationists as they face the prospect of defeat over efforts to introduce a radical new approach to flood control in the wake of the summer's Mississippi floods.

Whenever there has been a flood disaster in the United States, weighty reports have been produced and gestures made by government supporting a flood system based more on natural wetlands and less on river engineering and levees. But, as those attending a 300-strong workshop on post-flood recovery organized in St Louis last week by wetland and flood-plain managers' associations were told by Californian environmental campaigner Ann Riley, "our control system still looks just like it did in 1942".

Farmers like a no-nonsense approach to flood control, and the Army Corps of Engineers likes building levees. As the federal government plans its \$7-billion recovery programme for the Mississippi, alliances between the two at local level are already threatening to outflank the more conservationist strategy favoured by Washington.

Conservationists are hoping for support from those who live in cities and from the Clinton administration. One of the president's environmental advisors, Will Stelle, told the workshop that the administration is receptive to their ideas. "It's time to recognize that the systems we have in place are not good enough", he said.

The White House and the Office of Budget and Management have already set up a work-

ing group to look at the reconstruction of the levees built to hold back the Mississippi and protect the flood plain. And a directive was sent to all federal agencies in August instructing them to "keep alternative options open" as they deal with flood recovery.

According to Stelle, an interagency scientific assessment team is to be set up in a few weeks to oversee the recovery effort in the short term. And the White House has instigated a full review of the way in which rivers are managed whose outcome will set the tone for national policy for the next decade. But the window of opportunity for a change in the techniques of flood control is narrow, and some say it is already closing.

Gilbert White, 50-year veteran of the river restoration movement and founder of the Natural Hazards Center at the University of Colorado, says that critics of previous policies have about a year to achieve changes at the national level.

White has been pushing for a four-year moratorium on levee rebuilding, under which the federal government would promise to indemnify farmers for flood losses sustained during that period. But with the Mississippi already in flood again, and its saturated basin promising more trouble in 1994, that option looks expensive.

At its simplest, the conservationist case is that there are too many farmers living in the flood plain, and that the power of the river in flood is aggravated by levees built to protect crops. This power could be dissipated, they say, by restoring natural wetlands to absorb the water.

The farmers do not appreciate this point

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Last summer's floods: will the lessons be learnt

of view, and the tension between the two sides is palpable. "Most of us have never met a real environmentalist", one farmer told last week's workshop. "Give us a practical alternative to levee reconstruction. Tell us how we're gonna survive".

The suggestion that natural wetlands would lessen the impact of major floods is hotly disputed, and will be a central subject of the research undertaken in the wake of the Mississippi flood (see *Nature* 364, 747; 1993). According to the Army Corps of Engineers, wetlands can have a marked impact in the containment of small floods, but not on the "hundred year flood" that arrived this summer.

According to Eugene Stakhiv of the corps' Institute of Water Resources at Fort Belvoir, Virginia, large areas of wetland would have reduced the height of that flood by only two or three feet, as previous months of heavy rain would have saturated the wetlands and removed their ability to absorb floodwater.

The corps' position on the rebuilding of levees is, however, ambiguous. Its Washington-based headquarters is still promising restraint, pointing out that only 12 repairs have been completed and 44 approved so far out of 1,100 damaged levees. But local corps offices boast of their speedy action to restore the flood barriers. One corps official says privately, for example, that on the upper Mississippi proper, all 37 levees will be fixed before winter comes.

Most levees have been breached only in one or two places and can be restored speedily and cheaply. Under current regulations, only around 300 levees are eligible for repair. But intense pressure from farmers and politicians is serving to persuade local corps commanders that many more levees are eligible, and should be fixed. It is this process, rather than any White House policy pronouncement, that is making conservationists feel they are losing the ball game once again.

Colin Macilwain

Rhône-Poulenc lifts Institut Mérieux stake

Oxford. Rhône-Poulenc, the French state-owned chemicals and pharmaceuticals company which is soon to be privatized, is about to reinforce its position as the world's leading producer of vaccines by acquiring full ownership of the Institut Mérieux.

Rhône-Poulenc already owns 51 per cent of Institut Mérieux. Negotiations are now taking place to increase this to 100 per cent. Under the proposed deal, shareholders in Institut Mérieux will get 77 ordinary Rhône-Poulenc shares for every five Institut Mérieux shares they hold, valuing the outstanding stake at about US\$1.3 billion.

Institut Mérieux has played an important role in the development of vaccines technology, and was, for example, responsible for the production of the first European polio vaccine in the 1950 and the first commercial vaccine against meningococcal meningitis.

Its profitability and sales have increased in recent years, with new products such as paediatric vaccines against meningitis fueling the surge. New products are in the development pipeline, and access to Rhône-Poulenc's deeper pockets will help Mérieux cope with high development costs.

According to a company spokeswoman the transaction "will strengthen Rhône-Poulenc's health business in the strategically important and fast growing area of preventative medicine". The new arrangements are expected to enhance the synergism that already exists between the two concerns, although there are no plans to merge Institut Mérieux activities with existing Rhône-Poulenc ones. Nor will existing deals already signed by Institut Mérieux with other companies be affected by the proposed transaction.

Mark Ward

Large firms 'benefit most from EC grants'

Paris. The assumption that small companies are more innovative than big ones is challenged in an independent evaluation of the European Community's BRITE-EURAM research programme which has been carried out by the Bureau d'Economie Théorique et Appliquée (BETA) of Strasbourg.

The assessment looked at 176 companies and research organizations that took part in the programme, which provides support for research into innovative industrial technologies. It concluded that they expect to generate Ecu 413 million (US\$480 million) in product sales or cost reductions from the Ecu 39.4 million of research funding they have received. The evaluation also says that technology transfer, increased training and other "indirect" benefits will generate a further Ecu 132.2 million.

The BRITE-EURAM programme is only open to research projects involving at least two partners. Companies that paired up with basic research laboratories generated product sales and cost reductions estimated at five times the amount of funds they injected into a project, according to the report.

Marc-Jacques Ledoux, who led the study, says that this result contradicts theories which argue in favour of cutting public funding of fundamental research in order to benefit applied research. Such theories, he says, are based on the "nonsense" that basic research only creates revolutionary and not incremental innovation.

But the report also finds a big difference

in the value of BRITE-EURAM projects to firms of different size. Big firms generated Ecu 25.3 in product sales and cost savings from every Ecu of public funding; in contrast, small firms generated only 2 Ecu.

Ledoux says this result reflects that fact that small companies often lack the cash to develop and sell new products arising from their research. Big companies also enjoy the advantage, he says, of having all the competencies needed under one roof. Consortia of small companies did much better, for example, when they developed their own system of vertical integration by bringing together firms involved in research, product-development, and product-use.

According to the report, Britain earned almost twice as much per Ecu for its investment in BRITE-EURAM projects than Germany, six times more than France, and twenty times more than Italy.

One of the more surprising aspect of the

report is that BRITE-EURAM has already produced economic benefits for participating companies. Community funding rules specify that research must be "precompetitive" — far enough from the market to allow companies to share results without swapping trade secrets. Ledoux says that the notion of precompetitive research stems from an obsolete linear model of innovation.

A separate evaluation of the BRITE-EURAM programme, by the Programme of Policy Research in Engineering, Science and Technology (PREST) in Manchester, arrives at similar conclusions. Neville Reeves, who took part in the evaluation, says this is one reason that demand for BRITE-EURAM funding is outstripping supply. "If available money is likely to produce near term benefits then demand will go up and up and up."

Declan Butler

Biological survey needs more support

Washington. The National Biological Survey (NBS) set up in August by Bruce Babbitt, the Secretary of the Interior, lacks sufficient means to meet its goal of assessing every ecosystem in the United States, according to a report published this week by the National Research Council (NRC).

The report says that achieving this goal will require greater coordination of the biological research carried out in various agencies throughout the country.

The NBS is intended to bring together the biological research and survey work scattered throughout the Interior Department, and in particular that carried out by the Bureau of Land Management, the National Park Service and the US Fish and Wildlife Service (see *Nature*, **364**, 751; 1993).

Babbitt wants the survey to provide decision makers with better information and

techniques to manage the nation's biological resources. He also sees it as a way of averting clashes such as those between environmentalists and the timber industry over the fate of the northern spotted owl in the Pacific Northwest.

The NRC report says that the survey is an important first step in this direction. But it argues that the NBS alone cannot muster the research and information resources needed to fulfil its goal of compiling an inventory of every ecosystem in the United States.

The report recommends that the Department of Interior set up an accompanying programme to stimulate and coordinate biological research carried out by federal and state agencies, museums, academic institutions and non-governmental organisations. It says that this "National Partnership for Biological Survey" — which the NBS would lead — must be "science-driven".

One of the main functions of the partnership would be to provide information suitable for use by government regulators, legislator, planners and resource managers. The report recommends that the NBS abandon the idea of creating a large central database to hold such data. Instead it recommends that the partnership should link new and existing databases throughout the country, through standard interfaces such as Internet.

The report also says that the number of staff transferred to the NBS from within the Department of the Interior will not meet needs. It says NBS will need to hire more botanists, ecologists, invertebrate zoologists, population biologists, information scientists, social scientists, statisticians and taxonomists.

Diane Gershon

BIG FIRMS DO BETTER

Parameters	Big	SME
Number of firms	75	38
Ratio of direct effects ¹ /EC funding	25.3 (503.7)	2 (17.9)
Ratio of indirect effects ² /EC funding	5.45 (108.5)	2.75 (24.9)

SME = Small and Medium Enterprises

¹direct effects = sales/cost reduction

²indirect effects = technology transfer/improved networks, reputation, organization, etc./training

Figures in parentheses are for total MECU

Stanford wins in bid for B factory

Washington. A high-energy facility known as the B factory, designed to produce large quantities of B mesons through collisions between electrons and positrons, should be built at the Stanford Linear Accelerator Center (SLAC) in California, President Clinton has announced. SLAC won out against New York's Cornell University in a fierce contest to host the \$170 million facility. Despite a detailed technical assessment carried out during the summer by an independent panel chaired by Stanley Kowalski of Massachusetts Institute of Technology, the decisive factor in the choice was almost

certainly political (*Nature* **364**, 7; 1993).

Clinton announced the decision at a San Francisco press conference on his plans to bolster California's moribund economy. Now that a site has been selected, Congress must decide whether to appropriate money for the project in the coming financial year. A conference between the House and Senate is expected to do this within days: the same conference will determine the fate of two other contentious research programmes, the Superconducting Super Collider and the Integral Fast Reactor.

Colin Macilwain

UK-Japan agreement to boost research collaboration

Tokyo. Britain's Prime Minister John Major and his Japanese counterpart, Morihiro Hosokawa, have agreed to recognize the political importance of the growing scientific links between their two countries by drawing up an umbrella agreement aimed at encouraging even greater collaboration between their researchers.

The agreement was reached during Major's visit to Japan two weeks ago, which was immediately followed by a separate visit by William Waldegrave, Britain's science minister. A draft agreement is being drawn up, and officials hope the final version will be ready within a few months.

At present, although a number of individual agreements exist between scientific institutions in the two countries, there is nothing comparable to that between Japan and several other leading industrial nations, in particular the United States.

An attempt to draw up such an agreement was blocked ten years ago when Japan rejected a British proposal that it should cover industrial research. The new agreement will be confined to collaboration between institutions in the public sector, although it is hoped that it will stimulate private sector cooperation. Even without an umbrella agreement, collaboration is flourishing, to judge from an exhibition of a handful of collaborative projects visited by Waldegrave at the British Embassy in Tokyo.

The star exhibit was a display of some of the results of observations of the Sun by the Japanese satellite Yohkoh which carries a spectrometer built by a UK consortium led by Mullard Space Science Laboratory of University College London. The project has produced X-ray video images of unprecedented clarity of sunspot activity during the present solar maximum.

Other exhibits were of a more industrial nature. For example, a consortium of five Japanese companies (Hitachi, Nissan,

Yamaha, Bridgestone and MTT Instrumentation) is contributing £3 million over three years to a joint project between the University of Southampton and Tokyo Denki University to develop audio equipment applications of 'anti-sound' (the elimination of sound waves in a system by feeding in matching sound waves that are out of phase).

Waldegrave appears to have been particularly impressed by the joint development of information technology by the computer-maker ICL of the United Kingdom and Fujitsu, the Japanese company which is now a major shareholder in ICL. Nearly 20 British engineers from ICL have worked for two years at Fujitsu's laboratories in Kawasaki and Numazu, south of Tokyo, and a similar number of Fujitsu engineers have been assigned to ICL in Britain to develop products such as the 'intelligent pad', a software tool for the multiple display of data, video, and sound on computers.

Waldegrave said, in line with the British government's new policy of linking research to 'wealth creation', he is particularly keen to encourage collaborations that will result in the manufacture of new products in the United Kingdom. But much of the present collaborative research between Japan and Britain is of a more basic nature.

Minoru Oda, former president of the Institute of Physical and Chemical Research (RIKEN), who oversaw initial planning of the Yohkoh project when he was director-general of the Institute of Space and Astronautical Science (ISAS) in the 1980s, admitted surprise at a British Embassy dinner attended by Waldegrave at the speed at which collaboration in science has developed over the past decade.

There are now more than 130 joint research projects between universities and national research institutes in Japan and Britain. Notable among them are the collaborations with ISAS, such as Yohkoh, a \$5-million programme on materials science at the University of Cambridge and Imperial College, London, funded by the Research Development Corporation of Japan, and a £15-million investment by RIKEN in a pulsed muon source at the Rutherford Appleton Laboratory in Britain.

Many Japanese electronics and pharmaceutical companies are also supporting basic research at Britain's universities and government laboratories. One example on display at the exhibition is the £1 million per year being invested by Teijin, a Japanese textile manufacturer, in research at the Medical Research Council's Collaborative Centre in Mill Hill, London, into ways of regenerating nerves in the central nervous system.

David Swinbanks

Clinton moves to lift barriers on technology exports

Washington. Computer users around the world can expect easier access to US-built machines following the Clinton administration's announcement last week that it is lifting export licensing requirements on powerful personal computers, Unix workstations and small supercomputers.

The shift is one of a set of decisions designed by the administration to demonstrate its determination to take a more proactive role in working with US industry. Other developments announced by President Bill Clinton and commerce secretary Ron Brown included a 65-point strategy to bolster US exports of manufactured goods, and a \$1-billion-a-year joint research initiative with the motor industry.

Current rules stating that computers the size of a standard 486-based PC require a licence for export will be lifted, to cover only machines 20 times as powerful. This threshold will be doubled again, once the necessary agreement is negotiated with the United States' partners in Cocom, the Paris-based trade control body.

Additionally, the lower memory limit used to define a supercomputer will be raised by a factor of ten, reflecting recent technological advances. Such machines are subjected to stringent rules by the US Department of Commerce, including security guards during transit to prevent theft.

The changes have been warmly welcomed by the US computer industry, which says that they will save a considerable amount of money, although industry officials decline to say how much. In practice their impact will be restricted: the larger companies are allowed to apply for export licenses to their most important Western markets *en bloc*, and restrictions on sales to the former Soviet Union and China are unaffected.

In a separate move, Clinton joined the presidents of Ford, Chrysler and General Motors on the White House lawn to announce a "partnership" to work on manufacturing technology, short-term development and long-term research, with the objective of developing vehicles capable of travelling 80 miles to the gallon. The programme will be managed by a team led by Mary Good, undersecretary of technology at the Department of Commerce, but will not initially involve new government expenditure.

A spokesman for the US Council for Automotive Research, which will coordinate participation by the automobile companies, says "They've agreed to do a programme but they don't have a roadmap for it yet." The government currently spends about \$500 million a year on automotive research, compared to the \$11 billion spent by the industry itself. **Colin Macilwain**

Software engineer acclaimed

The National Academy of Engineering's Charles Stark Draper prize — said, at \$375,000, to be the richest in the engineering world — has been awarded to 69-year-old John Backus, who developed Fortran as the first-ever high-level computer language when working for IBM. Backus, who is now retired and living in San Francisco, is the third winner of the biennial award, which is intended to acknowledge "outstanding engineering achievement". Previous winners have been the inventors of the integrated circuit and the jet engine. □

Fraunhofer learns to tighten its belt

Munich. Germany's Fraunhofer Society — the publicly funded association of 48 applied research institutes — is hoping to benefit directly from the desire of the country's new research minister, Paul Krüger, to see a major shift in government funding from basic to applied research.

Earlier this year, the minister announced that the research budget of his ministry, the BMFT, will be frozen at its current level of about DM9.5 billion (US\$6.0 billion) for the next two years. Included in the freeze is funding for the Fraunhofer institutes, putting greater pressure on them to seek research contracts from the private sector.

But Hans-Jürgen Warnecke, who takes over as president of the society this month, says Krüger has told him in private that he would like to increase the allocation to the Fraunhofer institutes. These are the main recipients of government support for applied research, and the increase would be achieved at the expense of BMFT's support for basic research. At present, about 40 per cent of the BMFT budget goes to basic research. In public, Krüger has said that he intends to maintain this figure. But Warnecke

says Krüger would in fact like to see this fall to about one-third, and that there is backing for this view in political circles.

"For example, there are strong opinion leaders in parliament who say that if we can't have more money for research, it should be better distributed, that is with more emphasis on applied research", he says.

Warnecke, who is 59, is a professor at the University of Stuttgart. He has also been head of the Fraunhofer Institute for Manufacturing and Automation in Stuttgart, now the largest in the country, since 1971; Warnecke says that he plans to extend across the whole of the organization the "scientific entrepreneurialism" techniques he has developed at Stuttgart.

He takes over at a time when the institutes are feeling the first real pressure on budgets since the organization was set up in 1949 as part of German's post-war reconstruction. Last year the Fraunhofer institutes in the old *Länder* had their project funding from the BMFT cut by more than 20 per cent, to DM169 million, although their basic funding was held at DM176 million.

In a bid to make up the shortfall, the various institutes increased their joint income from private contract research from DM183 million in 1991 to DM191 million in 1992. Warnecke believes they will have do even better if they are to survive. But he also believes that the funding shift favoured by Krüger would help.

Warnecke is aware that competition to Fraunhofer institutes for applied research contracts in Germany is not strong at present. Most comes from universities and national research centres, which have much lower overheads than the institutes. But these are only effective in a few areas, such as information processing or laser development.

In future, says Warnecke, the competition is likely to increase. "But we have one thing that universities and basic research centres tend to underestimate", he says. "Our culture of result-orientated work means that, unlike these other institutions, we are used to working to tight time and cost constraints."

The Fraunhofer institutes have a high reputation. But Warnecke is not complacent. In a document *Leitbild 2000* (*Guidelines 2000*) to be published at the end of this month, he emphasizes that the society has a responsibility to develop applied research and development in the service of both industry and society, and is keen to increase the effectiveness with which it meets these goals by applying more market-orientated criteria to evaluate research. Scientists will be given more freedom and responsibility in choosing research projects. In return they will be expected to change research areas quickly in response to industrial and social needs. And their continued employment will depend on their ability to attract funds.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

**New president Hans-Jürgen Warnecke
wants more 'scientific entrepreneurialism'**

No immediate cuts are expected in research staff. But, says Warnecke, there will be a reduction in the 350 administrators at the society's headquarters in Munich, and more services will be decentralized. Central services that are retained will be made available strictly according to demand: for example, institutes requiring support for patent applications will pay for the service on an *ad hoc* basis.

Warnecke says he is determined to take tough action against institutes that are not sufficiently effective in attracting contract research funds. Those failing to meet an agreed target for such research will be evaluated by a committee of experts from both within and outside the Fraunhofer Society (One such investigation is already under way and will be completed this year).

Institutes failing to make the grade will be closed. "My style has always been to give scientists the freedom and responsibility they need, while not being afraid to sack those who don't come up to scratch", says Warnecke.

Alison Abbott

Fraunhofer to keep east German centres

Munich. The Fraunhofer Society (see story above) has decided to retain all of the institutes — and most of the research groups — that it established in the new *Länder* immediately after reunification for a three-year trial period.

Shortly after the Berlin Wall came down in October 1989, teams of experts were sent into east Germany to assess the research being done there. At the same time, the Fraunhofer Society began negotiations with the German research ministry (BMFT) to secure the necessary funding to take over the best research.

By 1990, ten institutes, employing 732 staff, had been set up on a trial basis. Seven of the ten will now be taken over fully from the middle of next year; the three others have another two years to run before a decision is made about their future.

In addition, 12 research groups — making up a total of 245 scientists and technical staff — were incorporated into institutes in the old *Länder* in what was formerly West Germany. From next year, ten of these groups be become full Fraunhofer members.

The Fraunhofer groups in the new *Länder* have not been slow in attracting industrial funding. Last year, according to the society, they received DM14 million from industry for contract research. **A.A.**

Army AIDS vaccine trial faces new block

Washington. An amendment passed in Congress last week could derail the US Army's proposed \$20-million clinical trial of the controversial gp160 AIDS vaccine produced by the Connecticut-based company MicroGeneSys (see *Nature* **364**; 751, 1993). The amendment to the defence appropriations bill, proposed by Henry Waxman (Democrat, California) and passed by the House of Representatives without a vote, gives the Army more time to agree details of the trial with the National Institutes of Health and the Food and Drug Administration.

AIDS activists say that such a renegotiation would effectively scupper the proposed trial, with the \$20 million it would cost reverting to other Army medical research. The Senate, which initiated the MicroGeneSys trial as part of last year's budget, has yet to give its opinion. **C.M.**

Nations urged to unite on drilling costs

Munich. Plans are being drawn up for an International Scientific Continental Drilling Programme (ICDP) which geophysicists hope governments will agree to support as a way of sharing the costs of drilling and operating boreholes in different parts of the world.

One project that could benefit from such an international programme is a proposal from US scientists to drill a hole of at least 10 km into the San Andreas Fault to study the mechanisms of earthquakes.

Supporters of the ICDP point out that, as well as revealing knowledge of the Earth's crust, drilling also provides information about natural phenomena which have a direct impact on society, such as the origins of earthquakes, mechanisms of climate change and possibilities for the safe geological disposal of hazardous waste.

But continental drilling, particularly at great depths, is both expensive and technologically challenging. As a recent report to the Megascience Forum of the Organization for Economic Cooperation and Development pointed out, there is therefore a strong case for international cooperation.

Scientists and representatives of funding bodies from 13 countries met in Potsdam last month to discuss how this cooperation might be achieved by the proposed ICDP. The meeting agreed that a science programme should be drawn up for such a programme by a committee of scientists from ten countries. This will form the basis for negotiations for funding from individual countries, as well as from potential international bodies such as the European Science Foundation and the European Communities.

The structure of the ICDP itself remains to be determined. Mark Zoback, professor of geophysics at Stanford University and a member of the ICDP committee, says that one possible model is the US-led Ocean Drilling Program (ODP).

Under the ODP model, any country wishing to participate would pay a basic fee to

cover administrative staff, the costs of drilling, and for some scientific projects. Research teams would then apply either individually or jointly for project money, and their applications would be assessed by expert panels.

Germany, which has long experience of scientific continental drilling, is expected to lead the ICDP. A national geological sciences research centre has been set up in Potsdam, and the centre has already offered at least one staff member to help develop the initial planning of the ICDP.

Germany began its own drilling programme, known as the KTB (Kontinentales Tiefbohrprogramm) 15 years ago at a site in northern Bavaria. The hole is now 8.3 km deep, and should reach 10 km by the end of the year. This is the depth at which the heat is such that rocks become plastic and are unable to transmit mechanical forces.

ICDP committee member Rolf Emmermann, who is both head of the geosciences research centre and a scientific director of the KTB, says that discussions are already taking place with the ministry of research about keeping the borehole open for a further two years as a deep-crust laboratory. One proposal is that scientists from other countries should be asked to pay to use the proposed laboratory for their own experiments.

According to Emmermann, an international programme is justified not merely on the basis of cost-saving but also because many of the questions that boreholes can answer are global both in nature and in their importance to society. If the ICDP takes off, Emmermann says he hopes that the German government would agree to release the KTB drilling equipment for other projects, such as that to drill into the San Andreas Fault.

In the past, deep drilling has been criticized by some geoscientists who use other techniques to study the Earth's crust on the grounds that it provides data about a very limited area at a very high price. The ICDP

is likely to cost at least US\$40 million a year. But Rolf Meisner, a seismologist at Kiel University in Germany, claims that it takes the public and political appeal of 'big science' projects, such as the proposal to drill into the San Andreas Fault, to generate this sort of money.

Alison Abbott

Institute will act as 'brain centre' of Japanese science city

Tokyo. Japan's International Institute of Advanced Studies (IIAS) opened last week in plush new surroundings in the hills between Osaka, Kyoto and Nara. The institute, intended as the 'brain centre' of the Kansai Culture and Science City that is slowly emerging between the three cities, is headed by Michio Okamoto, a former president of Kyoto University, and is modelled on the Princeton Institute of Advanced Studies in the United States.

For the past several years, the institute has been occupying temporary headquarters in Kyoto. Local prefectural governments and private sector institutions have now injected billions of yen into providing it with land and the new building. The institute now includes a Japanese garden where scholars can contemplate the future of science and society.

The Kansai science "city" that it will serve is at present just a handful of isolated institutes in the mountains. But the city is expected to have a population of nearly 400,000 people when completed next century after an estimated investment of US\$25 billion.

IIAS hopes to invite leading scholars from all over the world to carry out research, most of which will be theoretical, with experimental work being carried out separately in universities or research institutes. There will be three projects on the theme of "theoretical life science", and another project will focus on the concept of "safe science".

The founders of IIAS were initially successful in raising funds from the private sector and local governments in the late 1980s and early 1990s when Japan's economy was booming. But fund-raising has since run into difficulties because of the recession.

Annual interest from the existing fund is only about ¥150 million (\$1.5 million), but IIAS needs about ¥500 million a year to cover its operating costs. An institute official says that it has enough funds to last for about two years. Its survival beyond that will depend on its success in raising funds from government agencies and the private sector.

David Swinbanks

IMAGE
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France's Institute for Marine Research (IFREMER) last week launched its new research vessel L'Europe. The institute has chosen a catamaran design for stability and convenience when lowering scientific instrumentations directly into the water. L'Europe will carry out a wide range of missions, including the study of fisheries resources, and seismic and echosounding operations. □

O. Barbraud/IFREMER

Some you win, some you lose

SIR — I have long suspected that DREADCO has been less than forthcoming in its communication with David Jones. Now there is proof in Jones's description (*Nature* 364, 492; 1993) of DREADCO's development of Fluorogro[®], a fluorescent spray that doubles the rate of plant growth by reradiating as chlorophyll-absorbing visible light the ultraviolet wavelengths that Daedalus claims are reflected relatively well by chlorophyll. Actually, of course, it is near-infrared wavelengths that are reflected well by plant leaves, the ultraviolet wavelengths being reflected very poorly (Gates, D. W. *et al. Appl. Opt.* 4, 11–20; 1965). Nevertheless, just in case the thinning ozone layer has altered this well-known property of leaves, I performed a quick test with a radiometer equipped with channels at 315 nm in the ultraviolet and 1000 nm in the near-infrared. The leaves of a nearby cedar elm tree reflected only minuscule amounts of ultraviolet, while the reflectance at 1000 nm was, as expected, approximately 50 per cent.

The fundamental idea behind DREADCO's scheme does work, for there are fluorescent materials that reradiate visible wavelengths when illuminated by near-infrared. It can therefore be concluded that DREADCO is out to deceive its competitors by its misleading disclosure to Jones. I shall be glad to level the playing field by supplying the name of a source of infrared-sensitive fluorescent material, which I call FloiRogro[®], to DREADCO and its competitors, who are invited to send non-exclusive royalty offers to the address below.

Forrest M. Mims III

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SIR — Daedalus (*Nature* 365, 18; 1993) suggests that diving seals have a protein in their blood that inhibits bubbles from growing when they return to the surface, thus preventing them from getting 'the bends'. He further notes that the mechanism would probably be similar to that found in polar fishes, where antifreeze proteins inhibit the growth of ice crystals.

While I was working on fish antifreeze proteins in Antarctica a few years ago, I obtained some blood from the deep-diving Weddell seal from another research team that had a small surplus. I pressurized a few millilitres of gently swirling seal serum and Ringer's solution, a control, to 30 p.s.i. nitrogen gas at room temperature. After one hour (about the length of a good dive), I placed drops of the two liquids under a cover slide and

First anthropoid station at risk

SIR — The studies of problem-solving by chimpanzees conducted by Wolfgang Köhler on the Canary Island of Tenerife between 1914 and 1918 are historic. His work on the subject, *The Mentality of Apes*, is a classic and the picture in it of a chimpanzee angling for a suspended banana with the use of boxes and sticks has become a textbook cliché¹. Films, some made by Köhler at the time, are available for teaching. The observations played a role in the genesis of *gestalt* psychology, co-founded by Köhler, and provided ammunition in the continuing polemic on animal consciousness. The past decade or so has seen a renewed interest in Köhler, his apes and the first primate research station^{2–4}.

The site of the former anthropoid station lies on the northeastern edge of the expanding tourist resort of Puerto de la Cruz, next to a banana grove and within a few hundred yards of a shopping area and modern hotels. Köhler's unmarked house is tenanted and adjacent service buildings, in which apes were presumably quartered, still exist, but for how much longer is uncertain.

The land that includes the former station site is being offered for subdivision and the building of luxury apartments and houses as Phase 1 of "Urbanizacion La Costa" is being advertised in three languages. Construction machinery and material are on the station site.

The destruction of remnants of the



world's first primate research station would constitute a unique loss in the history of science — and perhaps potentially a loss to the tourist industry. Köhler's former residence, Casa Amarilla, still handsome though dilapidated, deserves preservation as an historic monument and commemoration by a plaque. Expressions of concern and support on the subject to the undersigned will be forwarded to authorities on Tenerife in a position to help bring about the preservation and recognition there of what is left of Köhler's Anthropoid Station.

H. S. Robert Glaser

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1. Köhler, W. *The Mentality of Apes* (Kegan Paul, London, 1924).
2. Ash, M. G. thesis, Harvard (1982).
3. Ley, R. *A Whisper of Espionage — W. Köhler and the Apes of Tenerife* (Avery, New York, 1990).
4. Lück, H. E., Jaeger, S. *Revista de Historia de la Psicología* 9 (2–3): 295–305 (1988).

looked at them under a microscope. DREADCO, which planned to market a synthetic version of the seal protein, will be disappointed to learn (as I was) that there was no visible inhibition of bubble formation in the seal serum.

James A. Raymond

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SIR — After years of waiting enthusiastically for my weekly issue of *Nature* to arrive and then struggling to understand articles in fields distant from my own (facial plastic surgery). I was pleased and surprised to see you address a topic in my own area (364, 106; 1993).

Daedalus recommends developing a "DREADCO" Wrinkle Blaster" that would penetrate the upper dermis and tighten the skin. This recommendation is in essence a form of dermabrasion in which all of the epidermis and various levels of the dermis are mechanically removed to improve skin texture. The epidermis then regenerates from the epithelium of the skin appendages. (Dermabrasion is best described as "sandpapering" versus the proposed "sand

blasting".) Various forms of freezing treatments, including solid carbon dioxide, have been used for skin treatments and in conjunction with dermabrasion.

The currently popular technique to perform wrinkle removal is chemical peeling. The deeper peels such as phenol and higher concentrations of trichloroacetic acid (TCA) go more deeply into the dermis and help remove the deeper wrinkles. Interestingly, the current trend is to perform more superficial peels with weaker acids such as glycolic acid which create an effect similar to the proposed "wrinkle blaster." We have noticed some long term "slow and subtle facial rejuvenation" in some of these patients.

Now that Daedalus has turned his theoretical mind to the important area of texture in the ageing skin, I would ask that he address the underlying tissues. A facelift is performed primarily not to remove wrinkles but to tighten the basic sagging of the underlying tissues which have lost elasticity with age. A procedure that would nonsurgically tighten these tissues would be a definite advance.

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Religious divisions

SIR — In any discussion of religion, the following argument seems to me to be incontrovertible. There are many religions in the world, with several of them (including almost all branches of Christianity and of Islam) claiming to be valid for all people at all times. Each has numerous adherents of the highest integrity and intelligence. These faiths contradict each other, and so at most only one of them can be right. Accordingly a huge number of believers must be wrong. Thus it is plain that the human mind is singularly liable to be mistaken on religious issues, whatever the depth of conviction, intelligence and integrity of the faithful.

The past as well as the present can leave no doubt that the variety of religions is a calamitously divisive force in human affairs. The less this factor is brought in, the better for all. This is especially incumbent on those working in a universal and global enterprise as science is.

To come to some specific issues, there are those who claim that Christianity provided the necessary background for science with its belief in an ordered universe. How do they account for the fact that for three-quarters of the Christian era the home of science was confined to the non-Christian parts of the world, such as China, India and the Islamic countries?

It is also sometimes claimed that Christianity differs from some earlier religions by its opposition to human sacrifice. How can that be claimed for a religion that for centuries gloried in the burning of heretics and of witches?

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SIR — Ralph Estling (*Nature* 364, 754; 1993) thinks it was unfair of me to criticize Brian Josephson's "concept of religion as an attempt to maximize human goodness" by pointing to the religion of the Aztecs and Carthaginians. I did this to avoid reference to current religions of the world but this has led to misunderstanding.

The major religions of the world are a product of social evolution and are constantly changing their character and spawning new sects. Some are fanatical enough to practise terrorism as a method of imposing their will on their enemies. The attempt to blow up the World Trade Center in New York by followers of Sheik Abdel Rahman is just one example.

Christianity, which may appear benign to a myopic, insular observer, has itself gone through phases where it dealt harshly and inhumanely with dissenters. The Inquisition, active between the fifteenth and eighteenth centuries, used methods later identified with Stalin's purges of his opponents in the 1930s. Thousands of

heretics and witches were burnt alive before the conclusion of this phase of church history. There is no guarantee that it won't happen again, as the fighting in Bosnia-Herzegovina shows.

The so-called 'ethnic' cleansing of Bosnia by the Serbs is a misnomer for an attempt by Greek Orthodox Christians to eliminate the Muslims in their midst through expulsion, murder and rape. It has nothing to do with ethnicity, for both sides speak the same language and differ only in their religion. It is more brutal than the expulsion of Jews from Spain by the Inquisition at the end of the fifteenth century, yet the supposedly Christian European states stand by, unmoved by any "human goodness" that their religion may have instilled in them.

John L. Martin (in the same issue) evidently believes, along with Josephson, that any religions not conforming to the "human goodness" criterion are pathological variants, not to be included in a discussion of religion. Let me remind him that in a set of observations in physics it is not permissible to eliminate any data from a dataset unless observational errors are proved to be present. To use an arbitrary criterion for determining which data are to be included in a dataset would be simply bad physics. Logically this is equivalent to discarding all observations that show insects to have six legs because Aristotle said they have four. What I have described, colourful or not, does not fall into the category of observational errors and must be dealt with in any rational discussion of religion.

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SIR — Michael Houlder's letter (*Nature* 363, 389; 1993) about Josephson's defence of a scientific analysis of religion was thoughtful and erudite. Religious beliefs are rationalizations for various behaviours. Customs and cultures are much the same and generally support the community in preference to the individual.

Humanity has, as one of its unique features, a (relatively) prolonged period of nurturing before a child matures to the adult stage. The child, in self-defence against siblings who demand the attention of parents (resources), is egocentric. Greater egocentrism leads to greater acquisition of resources and hence survival. This works up to a point. A world filled with egocentric adults would degenerate into anarchy, and populations would die out quickly.

Cultures (and with them, religions) developed to assist the child to evolve over a lifetime from supporting the self to supporting the community. Ideally, a balance

is achieved between concern for the self and concern for others. In reality, a whole spectrum of behaviours develops, with disasters such as that at Waco at one (rare) extreme. On the whole, behaviours that allow one to procreate and nurture one's offspring to adulthood are the ones that survive.

Theology and social sciences fit well together in this context. Anything that occurs within the Universe is natural to it, hence theology and natural sciences might be related but the fit is not as good.

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ICTP search

SIR — A search committee has been formed to find a successor to Professor Abdus Salam, the founding director of the International Centre for Theoretical Physics (ICTP) in Trieste, Italy. This is a uniquely successful institute for fundamental research.

Although Italy pays 90 per cent of the costs of running the centre, it is now administered by the International Atomic Energy Agency in Vienna; from January 1994, the United Nations Educational, Scientific and Cultural Organization (UNESCO) will take over.

I write as one who has benefited from the centre to emphasize the importance of this appointment and to remark that the centre may suffer if the new director lacks leadership qualities and cannot deal with Italian and international civil servants and diplomats.

Among other things, ICTP exists to mitigate the intellectual isolation of physicists in developing countries. For this reason, the new director should be a citizen of a developing country and a physicist by training. The appointment should not be in the gift of the Italian government, nor should it be made as a result of political pressure on UNESCO.

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The reason why

SIR — "Why should anyone care whether animals are conscious?" asks Sara Shettleworth (*Nature* 364 398; 1993). Er, well, one possible reason does come to mind. If it were widely believed that animals are "conscious", it might no longer be seen as acceptable practice to imprison and experiment on them. But of course no scientists would be influenced by considerations of that sort, would they?

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Abstract models in search of problems

Most models come into being as aids in the solution of real problems, but there is no reason why the process should not be inverted by the creation of models for which there are no problems — yet.

Just as paintings are artists' models of the visual world, so are physicists' models of the physical world imperfect representations of reality. Impressionism is permissible in both fields. To physicists (and in science generally) it may be more beneficial that a model should have heuristic virtues, or be suggestive, than that it should slavishly represent all the details of a system. But almost always, in physics as in art, a model is some kind of a representation of something out there. Painters usually give even their most abstract work a title, suggesting that they had something in mind when they made it. And in physics, it may be thought, a model that is a representation of nothing in particular would be of no value at all. By definition. For is not the purpose of a model to assist in the solution of some problem that has been specified?

That, it seems, is to reckon without people's ingenuity. Or, more correctly, there may be models in physics that are analogous to strictly abstract paintings in that they evoke both problems and the solutions of them. One such has just appeared. Cleverly, for an essay in abstract modelling, it combines two trains of speculation that have recently been famously successful: the use of the vertices of a regular lattice as an approximation to a continuum, and the use of geometrical mapping rules to represent the behaviour of chaotic systems.

This is how the argument goes. First, define a lattice, which for simplicity is merely a set of points along a one-dimensional line and let each point be occupied by a dynamical variable of some kind. That might, for example, be the energy of some component of a whole system, or its orientation relative to some direction or its speed. That means that there is a separate dynamical variable for every point of the lattice, say $x(j)$, where j is any integer between 1 and N , the total number of vertices in the lattice.

The next step in the definition is to make the dynamical variables chaotic using some nonlinear mapping rule to relate each $x(j)$ at one time to the same quantity at some later time. If, for example, it is supposed that time as well as space is discrete (or executed in jumps of fixed amount), then the dynamics of each sub-system may be represented by a rule defining each $x_{n+1}(j)$ in terms of $x_n(j)$, the value of the same quantity one time-step earlier.

At this point, there is little interesting to be said about the system as a whole — the collection of the N lattice points, each with its own subsystem. It is simply a collection

of nonlinear systems evolving independently of each other. How to tie them together into a single system? Obviously there must be some interaction between one subsystem and its neighbours, which obviously must depend on the value of the variables sited on them. And the principle must be that simplicity is best. So why not think of the variables on each lattice point as quantities of something, and suppose that if the amount of the something at any point exceeds some predetermined value, the excess simply spills over onto the next lattice-point in the chain?

That is the model constructed by Sudeshna Sinha and Debabrata Biswas from the Bhabha Atomic Research Centre at Bombay (*Phys. Rev. Lett.* **71**, 2010; 27 September 1993). The obviously missing element is the rule specifying the evolution of the chaotic sub-systems, which they take to be $x_{n+1}(j) = 1 - 2(x_n(j))^2$ for each lattice point in the system, with the convention that x lies in value between -1 and 1.

To establish the interaction between sub-systems, there must also be a rule for spilling over. The authors offer several versions. First they define some critical value of x , say x_c , which they take to be the threshold over which excess material spills. Then there are two choices: spilling only in one direction, or equally in both. And what happens if spill-over carries a neighbouring variable above the same critical value x_c ? Then there must be further spill-over, to more distant lattice sites, until all the lattice variables are less than or equal to the critical value. To make the system consistent, something spilled over from the ends of the lattice disappears from further consideration.

Inevitably, numerical simulation takes over at this point. Sinha and Biswas first populate their lattice with random values of x (within the allowed range), let the spilling over happen, the system settle down, and then execute a further iteration of the chaotic variables. And then carry on indefinitely, as computers allow. A further refinement (which helps to make some behaviour of the model interesting) is the possibility of adding random quantities at random lattice sites.

A few simple properties of the system stand out. If, for example, the threshold value x_c is less in magnitude than 0.5, the system will eventually reach a steady state in which each dynamical variable $x(j)$ has the same value as the threshold. The reason for that stems from the properties of the mapping relation, which implies that x_{n+1} is always greater than x_n whenever the latter is less than 0.5 in magnitude. That in turn

implies that there is always spillover from each lattice point, and always enough of it to ensure that each is filled to the level of x_c . Interest then turns to the nature of the spillover, which turns out to be much like the problem of avalanches on the sides of an artificial sand-pile formed by the addition of grains to the surface in the phenomenon called "self-organizing criticality".

The fun begins when the critical parameter is greater. Then spillover is less common — the mapping relationship does less to magnify the separate variables, while the threshold is higher. Then the simulations show surprisingly rich behaviour at individual lattice sites. Often there will be a cyclical variation of the variable at this site which is then interrupted by apparently random (or chaotic) motion. The periodicity is more pronounced at the centre of the lattice, more chaotic at the outsides. The size of the lattice seems to matter a great deal; the larger, the more pronounced the periodic behaviour of the lattice elements.

The temptation to replace "lattice elements" with the word "neuron" is bound, at that point, to be strong. If Sinha and Biswas's construction is a model looking for a problem to which it may apply, might it be a way in which groups of uninstructed neurons could organize themselves into coherent function? The authors themselves refer to the possibility that the coordination of synapses might be in such a way.

Other connections readily suggest themselves. The model now described, for example, has much common with the simulation technique of the cellular automaton (conceptually due to J. von Neumann). There again there is an array of lattice elements evolving separately in time, but according to rules that imply interaction among them. And again the result often seems to be a delicate mixture of order and chaos. Indeed, it seems the difference between the two schemes is simply that of Sinha and Biswas allows more delicate tuning of the timing of the interaction among the lattice elements.

It is readily imagined that the publication of these simulation will send other practitioners rushing to their own machines. There is endless scope for tractable elaboration, which is a virtue. But what can be said about the analogy with the behaviour of neurons, for example? Sadly, there is a sense in which the modelling puts the cart before the horse. It would be different if somebody had begun with the problem and then built the model. The equivalent of abstract art may have no place in physics.

John Maddox

Opening the gateway

A. Klug

ON pages 512 and 520 of this issue^{1,2}, two crystal-structure papers report a decisive advance towards understanding the mechanism of the initiation of transcription. The papers describe the three-dimensional structures of complexes of the TATA-box binding protein (TBP) with the TATA-box itself, the familiar DNA sequence forming a core promoter element in eukaryotes (and also a major element in prokaryotes). The results in both cases, although for different species of both protein and DNA, are very similar. They show dramatic changes in the conformation of the double helix, the like of which have never been seen before in any DNA-protein complex.

Initiation of gene transcription by RNA polymerase requires, in addition to the enzyme, the action of a number of general factors which form a specific multiprotein complex near the transcriptional start site by interacting with the core promoter elements (reviewed in ref. 3). Eukaryotes use three RNA polymerases according to the class of gene being transcribed, but they have much in common, including some of the auxiliary proteins.

The first step in formation of this pre-initiation complex in genes coding for proteins involves the binding of transcription factor IID (TFIID) to the TATA box, after which at least five other general factors bind. TFIID itself consists of a universal TBP and a fairly large number of associated factors (TAFs) which bring in the necessary species- and cell-specific variations. TATA-box binding protein alone binds with high affinity to the TATA box, and works with all three eukaryotic RNA polymerases. It does vary between species, but it has a 180-amino-acid carboxy-terminal domain which is highly conserved phylogenetically, as is, of course, the TATA-box, whose consensus is TATA_T/aA_t/aA. Now, thanks to much biochemistry and the power of X-ray analysis, a dozen or so years after the initial discovery of TFIID by Roeder⁴ we can see how TBP — the protein at the heart of TFIID — opens the way to transcription.

The paper by Y. Kim *et al.*¹ (the 'Yale group' of P. B. Sigler, with a contribution from Seattle) describes the structure of the complex of the carboxy-terminal domain of yeast TBP with a TATA-box element from the *CYC1* gene of yeast (Fig. 1a). That by J. L. Kim *et al.*² (the 'Rockefeller group' of S. K. Burley) is on a heterologous complex of TBP from the plant *Arabidopsis* with the TATA-box of the adenovirus major late promoter (Fig. 1b), which has been much used in the

biochemical studies by Roeder's group. The striking similarity in the detailed structure of both complexes (at about 2.2–2.5 Å resolution) speaks for the universality of the TBP–TATA interaction in eukaryotes, and defines, at last, the TATA-box element in structural terms as an eight base-pair sequence (boxed in Fig. 1).

The paper by Burley and colleagues follows their earlier determination of the structure of *Arabidopsis* TBP alone⁵, which revealed a quite novel protein fold for binding DNA. The structure of TBP

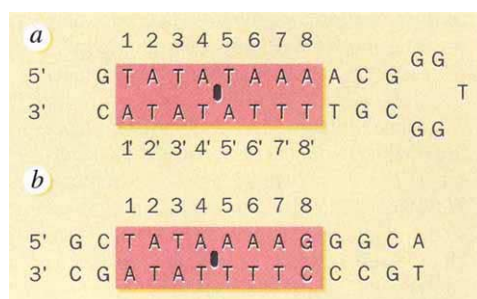


FIG. 1 Oligonucleotides used for co-crystallization with the TATA-box binding protein (TBP) by, a, the Yale group (Y. Kim *et al.*¹) and, b, the Rockefeller group (J. L. Kim *et al.*²).

consists of a curved ten-stranded anti-parallel β -sheet, generating a saddle-shaped structure, with four α -helices lying on its upper surface (Fig. 2, top). The molecule has approximately twofold symmetry, consisting of two subdomains which correspond to the two direct repeats in the amino-acid sequence. On either side of the saddle are two loops each formed by an anti-parallel pair of β -strands, giving the appearance of a pair of stirrups — each carries a pair of invariant phenylalanine residues which were known by mutagenesis studies to be crucial to the binding to DNA. Likewise other residues on the underside of the saddle were known to be involved in the specific interaction with the DNA (for references see ref. 5).

The dimensions of the molecular saddle of TBP are such that its concave surface could sit nicely astride a DNA double helix, so it was assumed that this was the case. However, the structures of the complexes^{1,2} show that the geometry is quite different — in fact the long axis of the protein saddle is not at right angles to the DNA axis, but nearly parallel to it (Fig. 2). This different orientation was foreseen by Kornberg and colleagues⁶, who very recently solved the structure of the yeast TBP and proposed that it wraps around the DNA in the minor groove, consistent with earlier chemical footprint-

ing studies⁷. In fact, even this suggestion did not go far enough — none of the groups, reasoning from the structure of TBP alone, envisaged the unprecedented degree to which DNA is bent and untwisted in its contact with the protein, which remains largely unchanged in structure except for a small change in the angle between the two subdomains². Indeed, as a result, the trajectory of the DNA nearly follows the arc of the underside of the protein saddle, at an angle of about 20°. Thus the TBP molecule is no simple saddle; rather it is a former, moulding in its vice-like grip the shape of the transfigured DNA to the curve of its concave surface.

The crystal structures of the two complexes both show a region of eight base-pairs interacting closely with the protein and deviating greatly from the β -form double-helical structure. One can, therefore, define the recognition motif for TBP in structural terms as an eight base-pair TATA element. The interaction with the TATA element causes the DNA to bend (writhe) through an angle of about 80° (Fig. 2) and, in consequence, the TATA element unwinds considerably, by about 110°. These two changes in writhe and twist almost exactly compensate for each other in topological terms, so that there is no net change in linking number, as indeed had been predicted from the observation that TBP bound linear

and circular DNA constructs with similar affinity⁸. (For comparison, imagine the untwisting of a twin-flex cable, held at both ends, in response to bending: the number of times the two strands wind around each other — the linking number — remains unchanged.)

To understand in detail how this is achieved, the original papers must be read. But, put briefly, the minor groove of the TATA element is widened and flattened, and interacts with the whole concave underside of the molecular saddle, making a variety of hydrophilic, and a surprisingly large number of hydrophobic, contacts. The curvature of the DNA is produced by large positive roll angles at all seven steps in the element, with especially large roll at the first and last steps (1 to 2 and 7 to 8 in Fig. 1). There the deformations are so large that they are better described as kinks; each is produced by the pair of invariant and essential phenylalanine residues emanating from each stirrup, which wedge between the adjacent base pairs and pry them apart.

What implications does this large change in DNA structure hold for the mechanism of transcription? Clearly, the bending at the TATA element could bring other initiation factors closer together than on linear DNA. The unwinding of the TATA element could well be a nucleation step for further unwinding towards

the start site, and thus for the eventual separation of the two DNA strands required for the initiation of transcription. However, since the Watson-Crick base pairing is still maintained in the unwound TATA element, and since the DNA structure returns abruptly to β -form conformation outside the element, interactions with other factors must be required for the unwinding to propagate further. It is easier to imagine this migration in prokaryotes where the distance between TATA-box and start site is only 10 base pairs, in contrast to 30 in eukaryotes. Whatever the subsequent events in the formation of the TBP complex, there is little doubt that in these changes in the TATA element we see the opening of the gateway to transcription.

But even before the addition of subsequent components of the pre-initiation complex can be considered, there is an important question to be answered about the TBP/TATA-element complex itself—namely, how is its polarity of formation achieved? The TBP molecule is approximately twofold symmetric and the direct interactions in the interface with the TATA element are all matched in detail in the two protein subdomains^{1,2}, even though the two ends of the TATA element are not symmetric in sequence (Fig. 1). Yet the polarity is such that the carboxy-terminal subdomain contacts the 5' end of the TATA element (that is, the invariant TATA sequence itself) and the amino-terminal subdomain interacts with the 3' end, which varies between different genes. There are subtle differences in the environment of the two halves of the TATA element (the Yale group draw attention to a set of lysines in one subdomain), which might give a preferred orientation, but a further and attractive possibility is an asymmetry in the deformability of the TATA element. The choice would then be made kinetically.

It was noted many years ago⁹ that the T-A step in a double helix is relatively unstable, and therefore easily deformable, and that this may account for the widespread use of TATA or TATA-like (or more generally pyrimidine-purine) sequences in a number of processes that involve DNA strand separation¹⁰. Indeed the more general point can be made that DNA need not be a passive participant in its interaction with proteins and that special DNA sequences can, through their physical properties, carry another, physical, level of information secondary to 'chemical' recognition between individual interacting groups¹⁰. In this case TBP exploits the deformability of the TATA sequence to initiate unwinding of DNA as a prelude to transcription.

Of course, if the polarity of TBP with respect to the TATA element were not to be determined directly by their interaction—and there are mutagenesis data to say

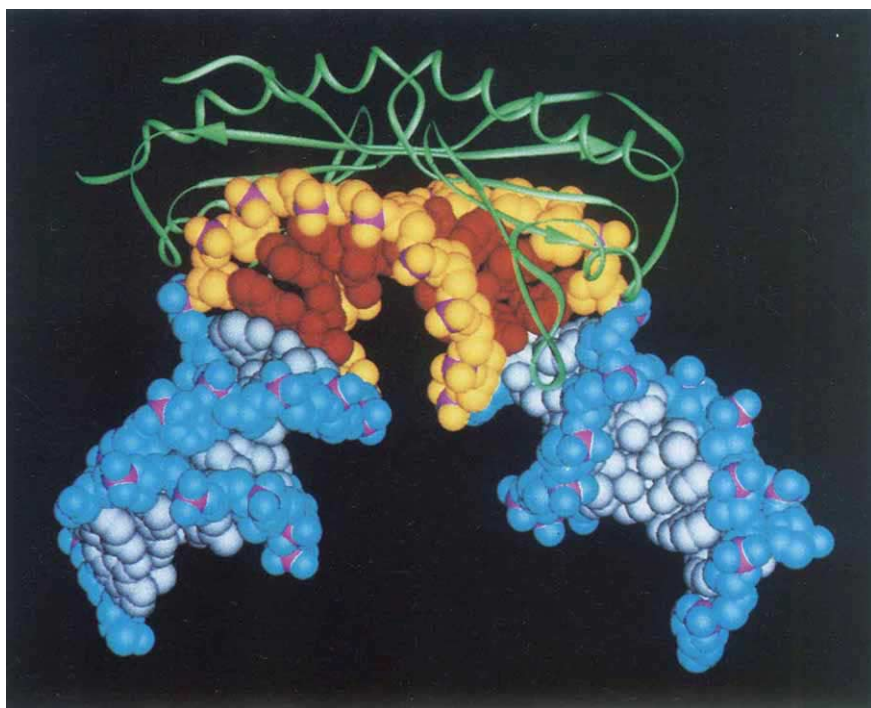


FIG. 2 The overall structure of TBP/TATA-element complex with computer-generated β -DNA extensions (in blue) at both ends (courtesy of P. Sigler). The saddle-shaped protein, which is approximately two-fold symmetric, is viewed with its long axis in the plane of the page and is represented as a green α trace: note one of the 'stirrups' appearing as a loop on the right-hand side. The phosphate-sugar backbone of the DNA is in yellow and the eight base pairs of the TATA element (whose 5' end is on the left) in red: the double helix is unwound to give a ladder-like appearance and its axis is bent into a right-handed supercoiled arc. The two β -DNA extensions are at angles of about 25° out of the plane of the page, with their ends pointing inwards on the right side and outward on the left (as can be seen from the cusps at the sides of the helices). The angle between the extensions is about 100°, so that the DNA is bent through 80° at the TATA element.

that it is (for references see refs 1 and 2)—it would certainly be determined at the next level of assembly of the pre-initiation complex, which must be oriented correctly with respect to the start site. A wrong starting polarity would simply lead to a non-productive complex. The elucidation of the three-dimensional structure of the pre-initiation complex must remain the ultimate goal of structural analysis, if the mechanistic (and probably many of the regulatory) aspects of transcription are to be understood. This would be an immense task, because it is clear that what is involved is some kind of transient 'transcriptosome', not less complex than a ribosome or replisome. But I do not doubt that with sophisticated biochemistry and genetics, and the power of X-ray analysis, it will be achieved one day.

In the meantime there is much to do. While the underside of TBP wreaks its dramatic changes on the TATA element, the upper convex surface of the saddle provides, either directly or indirectly, a target for upstream regulatory factors^{1,2,5,11}. The latest example can be found on page 562 of this issue¹², where it is shown that an E1A-like factor in embryonal carcinoma cells switches on a retinoic-acid dependent transactivation

pathway by interacting with specific regions on the convex surface of TBP. The old adage is that knowledge of the three-dimensional structure of a molecule almost always gives insight into the processes in which it is involved. The results from the Rockefeller and Yale groups are a splendid illustration of that point. □

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Catalytic molten globules

William F. DeGrado

FOR enzymes to achieve their remarkably efficient catalytic activity, their constituent polypeptide chains must be properly folded so that the active functional groups can interact. As yet, nature is far better at designing enzymes than any human biochemist, and although peptides with enzymatic activity have occasionally been assembled from first principles^{1,2}, their three-dimensional structures and mechanisms of action have remained something of a mystery. On page 530 of this issue, Johnsson *et al.* go one better³. For their synthetic polypeptide, which they dub 'oxaldie 1', they know the chemical make-up (14 amino acids long, mostly leucine and lysine) and the form of the folding (an α -helix) as well as the catalytic activity.

Peptides consisting of leucine (Leu, or

L) and lysine (Lys, or K) have a history of use in the development of models for protein folding and enzymatic catalysis. Early work^{4,5} showed that the conformation of Leu/Lys polymers depended on the order of the amino acids. Those with a repeat unit $(LK)_n$ folded into the flat zigzags known as β -sheets, whereas $(LK-LL)_n$ polymers formed α -helices.

Later investigations⁶ focused on short peptides with the residues arranged so that they roughly matched the period of an α -helix, $(LKLLKL)_2$, or a β -sheet, $(LKLKLKL)$. As monomers in dilute aqueous solution, these peptides have unordered conformations. But

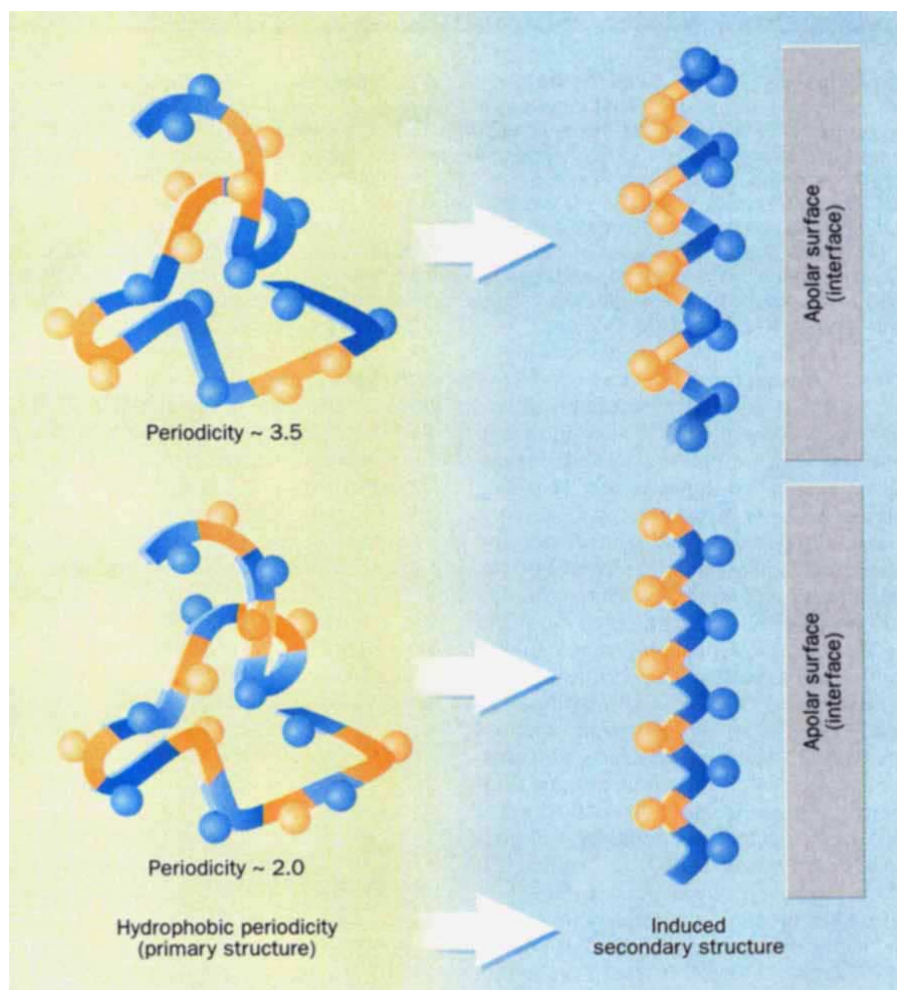
where there is an interface between water and a nonpolar solvent, the peptides bind to the interface as an α -helix or β -sheet. The driving force for adopting an ordered conformation, known as secondary structure formation, seems to come from the dehydration of apolar Leu side chains which occupy one face of the helix or sheet (see figure), as the ordered structure

Helical Leu/Lys peptides	
$(LKKL)_n$ polymer	L-K-K-L-L-K-K-L-L-K-K-L-L-K
$(LKLLKL)_n$	L-K-K-L-L-K-L-L-K-K-L-L-K-L...
Oxaldie 1	L-A-K-L-L-K-A-L-A-K-L-L-K-K
Mutant 1	L-A-K-G-L-K-A-P-A-K-L-G-K-K

exposes more of the Leu side chains to the apolar surface. $(LKLLKL)_2$ also self-associates to form α -helical oligomers; the concentration dependence of the circular dichroism spectrum⁶ seems to indicate that these are tetrameric. So it seems that Leu/Lys peptides are the simplest system capable of forming a protein-like conformation with a hydrophobic interior, a water-soluble hydrophilic exterior, and stable secondary structure. All the same, these and related peptides lack the specific electrostatic, hydrogen-bonded and van der Waals interactions that are necessary to stabilize a unique protein conformation. Instead, they apparently adopt more loosely bundled structures with fluctuating side-chain conformations, rather like molten globules^{7,8}.

Amphiphilic Leu/Lys peptides have a number of interesting functions such as potent membrane-perturbing activities⁹ and calmodulin-binding activities¹⁰. Other Leu/Lys peptides catalyse the hydrolysis of RNA^{11,12}; in some experiments, $(LKKL)_n$ accelerated the rate 100-fold, whereas a Leu-Lys dipeptide was inactive. A few years back, Benner and co-workers found that oxaldie 1, a 14-residue amphiphilic Leu/Lys peptide (see table), catalyses the decarboxylation of oxaloacetate (hence the name oxaldie 1). Oxaldie 1 accelerates the rate of this reaction by one to three orders of magnitude, depending on the standard of comparison. Like $(LKLLKL)_3$, oxaldie 1 apparently forms α -helical tetramers; the secondary structure was confirmed by NMR spectroscopy.

A difficult problem in assessing enzyme models is to determine their activities relative to some well-defined standard. Because the proper folding of an enzyme is essential for its catalytic properties, it appears that the best standard would be the enzyme model in an unfolded state. This is often difficult to achieve with the original structure, and analogues often fail to fold and provide good standards for



The ordering of the amino acids in a Leu/Lys peptide determines its secondary structure at an interface between an apolar surface and water. Leu residues are shown in blue and Lys in orange. The peptides, which are in random coil configuration in aqueous solution, here adopt an ordered (secondary) structure with a repeat matching the sequence repeat of the peptide. Amphiphilic secondary structure can be stabilized through interactions with apolar surfaces, such as the interface with air or through association with other amphiphilic secondary structures⁶.

comparison. For instance, substitute a Pro (P) for one of the Leu groups in (LKKL)_n, and the result is a randomly coiled (PKKL)_n polymer with 25-fold lower ribonuclease activity. Similarly, the random coil analogue of oxaldie **1**, mutant **1** (see table) is a 20-fold less active decarboxylase. These rate accelerations are a far cry from the rate enhancements of 10⁸ and greater observed for native enzymes¹³.

How do the peptides work? In the case of (LKKL)_n, the authors suggest that the lysyl amine groups serve both as general acids and general bases in catalysis, as well as being important for electrostatic interactions with the negatively charged RNA. However, they do not have the mechanistic or high-resolution structural information to confirm this. Oxaldie **1**, whose design derives from earlier studies of small molecules, stands on somewhat stronger mechanistic ground. Simple amines catalyse the decarboxylation of α -carboxyketones through a nucleophilic mechanism involving a Schiff's base intermediate¹⁴. Benner's new work suggests that the α -amino group of oxaldie **1** is the active nucleophile as an N-acetylated version of this peptide is significantly less active. Further, a Schiff's base intermediate was spectroscopically observed and trapped by reduction with NaBH₄. So the basic features of the proposed mechanism have support, if they are not proven.

The rate acceleration of oxaldie **1** seems to come from two sources, both of which require folding of the peptide: first, it contains an amine with a pK_a that is lowered through electrostatic interactions with the helical dipole and the polycationic side chains; second, it electrostatically attracts its substrate, a dicarboxylate.

What have we learned from these studies? The observed rate enhancements are modest compared with natural enzymes, suggesting that designer 'enzyme-

like' proteins will require greater functional-group diversity and structural definition. Further, all steps of the reaction mechanism must be catalysed; otherwise one might accelerate the most unfavourable step in a reaction sequence only to find that a second reaction or interaction becomes rate-limiting. Nevertheless, these catalysts do show activities that could be improved on, with appropriate selection. It is tempting to speculate that modern-day enzymes evolved from molten globule-like proteins, this state providing a plastic medium in which random mutations might have given rise to beneficial enzymatic activities. Mutations that increased the rigidity of the protein could then have locked the cata-

lytically active conformation in place.

A final note. The successful design of mimics of chymotrypsin and trypsin has just been reported¹⁵. Short peptide sequences taken from the active sites of these proteases are strung together between oligoglycine loops, and the resulting peptides are apparently nearly as active as the cognate enzymes at catalysing alkyl ester and peptide bond hydrolysis. If subsequent work confirms the findings, this very different approach might be a faster route into catalytically active peptides. □

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ATMOSPHERIC DYNAMICS

Mixing and matching

Alan Plumb

THE tropical stratosphere consists of 'young' air, recently upwelled from the lower atmosphere, containing fairly high concentrations of tropospheric source gases. Nearer the poles, on the other hand, the air is 'old', having downwelled from high altitudes where it has been transformed, in the harsh radiative environment, into a very different chemical mixture. On pages 533 and 535 of this issue two new studies^{1,2}, one based on satellite observations and the other on numerical modelling, give us a direct picture of the fluid dynamical processes by which these two different air masses are mixed together.

The meteorology of the winter stratosphere is dominated by the cold polar vortex and the associated 'polar jet' of strong westerly winds at the vortex edge. The vortex is surrounded by the mid-latitude 'surf zone'³, a region of large-scale, almost horizontal, turbulent motions. The surf zone is sustained by the action of Rossby waves, planetary-scale meteorological disturbances that are generated in the lower atmosphere and propagate up the dynamically elastic vortex edge. In the stratosphere these waves frequently reach large amplitudes and break, rather like ocean waves on a beach.

The breaking process strips tongues of air from around the vortex edge into the surf zone, producing a sharper edge that is dynamically more robust than before⁴. By midwinter, the sharp, robust edge is believed to act as a partial barrier to mass exchange. This barrier is semi-permeable, however, with tongues of air occasionally being extruded from the vortex. Although intrusions into the vortex have also been reported⁵, these apparently produce little mass transport, except during violent 'warming' events in which the vortex is

torn apart.

In qualitative terms, transport processes in high latitudes of the winter hemisphere have thus become reasonably well documented, but the rate of transport into and out of the vortex remains the subject of considerable debate⁶.

Fuel has recently been added to this debate with the publication of results from the HALOE instrument on the Upper Atmosphere Research Satellite (UARS) showing extensive dryness of the Southern Hemisphere lower stratosphere in spring⁷. The conventional explanation of stratospheric dryness is that air is 'freeze dried' as it passes up through the cold tropical tropopause. Although a tropical pool of dry air is evident in the HALOE data, a second pool of even drier air is located over the Antarctic, evidently a consequence of dehydration within the cold polar vortex during winter. By analysing the distributions of (2×methane + water), which is a diagnostic of Antarctic (rather than tropical) drying, Tuck *et al.*⁷ showed that most of the mid-latitude dryness must have come from the vortex, rather than the tropics. Thus, they estimated a timescale of 30 days for flow through the Antarctic vortex. This is difficult to reconcile with a value of 100 days estimated from dynamical considerations for the Arctic vortex⁸, given that all other evidence leads us to believe that transport in the Arctic is more vigorous than in the Antarctic, unless most of the dry air leaks out of the bottom of the vortex (at around 16 km) below which the transport barrier seems to disappear.

The surf zone is a stratospheric manifestation of a phenomenon known to fluid dynamicists as a 'nonlinear critical layer', the theory of which tells us⁹ that there should be a well-defined boundary on its

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equatorward side similar to that at the vortex edge. A partial barrier in the subtropics¹⁰ would inhibit exchange of air between mid-latitudes and equatorial regions. What mass transport occurs across this edge will probably arise from entrainment of tongues of tropical air into mid-latitudes, with relatively little transport of midlatitude air into the tropics.

Evidence has been accumulating to confirm this picture. Satellite measurements of ozone¹¹ and of volcanic aerosol from the eruption of Mount Pinatubo¹² have revealed tongues of tropical air being transported deep into mid-latitudes, while observations of the debris from nuclear tests in the tropical atmosphere¹³ and of volcanic aerosol from tropical eruptions^{14,15} show that a reservoir of such material survives in the tropics for two years or more, confirming that the tropical stratosphere is isolated from dilution with mid-latitude air.

Randel and co-workers¹ now add direct evidence of this behaviour. Their observations, from the MLS and CLAES instruments aboard UARS, illustrate both the entrainment of tropical tongues into the surf zone, another manifestation of Rossby wave breaking, and the existence of the subtropical barrier (made visible by the belt of strong material gradients in these latitudes). As the entrainment of dry, tropical air into the Southern Hemisphere takes place in winter and spring, it may well, as the authors suggest, make some contribution to the dryness evident in the HALOE data. It cannot, however, explain the substantial mid-latitude signal in ($2 \times$ methane + water) that is characteristic of Antarctic air.

Both the barrier and the tongues show up in distributions of the trace species nitrous oxide and water vapour, and also, more surprisingly, of potential vorticity, a dynamical tracer derived from global meteorological analyses³. The latter pattern is remarkable because very few direct observations of wind, needed to calculate potential vorticity, are available at these levels. Instead, we have to calculate winds

by assuming them to be in balance with the mass distribution, an assumption known to be suspect in the tropics (the lack — or perceived lack — of reliable tropical wind information has long hampered studies of transport in the tropical stratosphere). Nevertheless, the potential vorticity calculations seem to show that the calculated winds are useful, even within 10 degrees of the Equator.

Waugh's numerical results neatly confirm this². His calculations track material contours by advecting them with the same calculated winds; the contours reasonably reproduce the tracer patterns evident in the satellite data. These high-resolution results go beyond what is visible in the data to indicate that the ultimate fate of the tongues may be to be stretched, by the strong horizontal and vertical shear on the equatorward side of the polar jet, into long filaments around the vortex edge, closely intermingled with similar filaments of air ejected from the vortex. This pro-

cess by which the tropical and polar air masses are brought together is very different from the continuous diffusion assumed in models used to predict the future state of stratospheric ozone¹⁶.

Rapid progress is evidently being made in understanding how the mid-latitude stratosphere interacts with the tropics as well as with the polar vortex.

The big piece that remains missing from the stratospheric transport puzzle is: what happens within the tropics? Does, for example, air upwelling from the equatorial tropopause do so without significant dilution by mid-latitude air? We can expect further results from UARS, and from *in situ* aircraft observations, to help fill in the gap. □

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NUCLEIC ACID RECOGNITION

Marriage of convenience

Dinshaw J. Patel

A GROUP of Danish scientists has been at the forefront of research to develop oligonucleotide-based antisense and antigene agents as drugs against viruses and cancer. The approach they have pioneered centres on peptide–nucleic acid chimaeras (PNAs), and on page 566 of this issue¹ they report their latest results. Notably, they find that PNA with the four natural bases can form Watson–Crick duplexes with complementary DNA or RNA sequences. This is a step in the right direction along the long road that may end in the design of effective antisense drugs.

Such agents have to meet several initial criteria. They must be able to enter and be retained as stable entities in the target cell and must bind with high sequence specificity and affinity to their nucleic acid targets². To this end, analogues of oligonucleotide-based therapeutic agents have been synthesized in which the modifications range from localized substitutions of one or more atoms to the replacement of the entire ribose–phosphate backbone by its peptide counterpart. These are PNAs, and the most successful example has involved the replacement of the chiral and charged ribose–phosphate backbone (*a* in the figure) by a structurally homomorphous but achiral and uncharged polyamide backbone consisting of (2-aminoethyl)glycine units (*b* in the figure)³.

The chimaeric PNA that results is a marriage of convenience in which the recognition elements project from an uncharged scaffold. The PNA architecture

was designed by keeping it homomorphous with DNA and this was achieved by retaining the original number of backbone bonds in the repeating unit (six), as well as the number of bonds linking the backbone to the base (three)³. It turned out to be an excellent choice because a computational study has established that the PNA backbone adopts a helical conformation stabilized by an intramolecular hydrogen bond between the backbone amide proton and the side-chain carbonyl of the preceding residue⁴. The potential of this approach was limited in earlier studies to PNAs containing pyrimidines^{3,5,6}. But it expands dramatically with the description by Egholm *et al.*¹ of the synthesis of PNAs containing all four natural bases and the characterization of their complexes with RNA and DNA.

The stage was set for the new work by earlier investigations of interaction between homopyrimidine PNA and nucleic acids, and of the ability of these complexes to block transcription and translation at the PNA binding site. PNA homooligomers such as H–T_{*n*}–Lys–NH₂ (where *n* is 6, 8 or 10) were prepared with the carboxy-terminal lysine incorporated to increase solubility and reduce self-aggregation in solution^{3,5}. The (H–T_{*n*}–Lys–NH₂)–(dA_{*n*}) PNA–DNA oligomer complex was unusually stable relative to the (T_{*n*})–(dA_{*n*}) DNA–DNA oligomer duplex, with an increase in melting temperature of 10 °C for each added molecular unit. The stoichiometry of the complex was (PNA)₂–DNA, indicative of triple

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helix formation. Studies with thymine- and cytosine-containing PNAs demonstrated that the triplex was stabilized by T·AT and C⁺·GC triples at acidic pH (ref. 6), as has been reported for pyrimidine-purine-pyrimidine DNA triplexes⁷. Molecular mechanics computations on the homo-oligomeric (PNA)₂-DNA triplex suggested that the two PNA strands are antiparallel to each other, and that van der Waals attraction between the two nonpolar PNA peptide strands contributes to the stability of the (PNA)₂-DNA triplex relative to PNA-DNA duplex formation⁴.

Other studies of PNA T_n oligomer binding to poly(dA)·poly(dT) have shown that binding occurs following PNA strand invasion of the duplex through D-loop formation, resulting in two PNA T_n oligomers bound through Watson-Crick and Hoogsteen alignment to the poly(dA) strand in a right-handed triplex^{8,9}. This complex (c in the figure) could be imaged by electron microscopy, and was characterized further by gel electrophoresis which established helical unwinding of one turn of duplex per ten base pairs associated with strand displacement⁸.

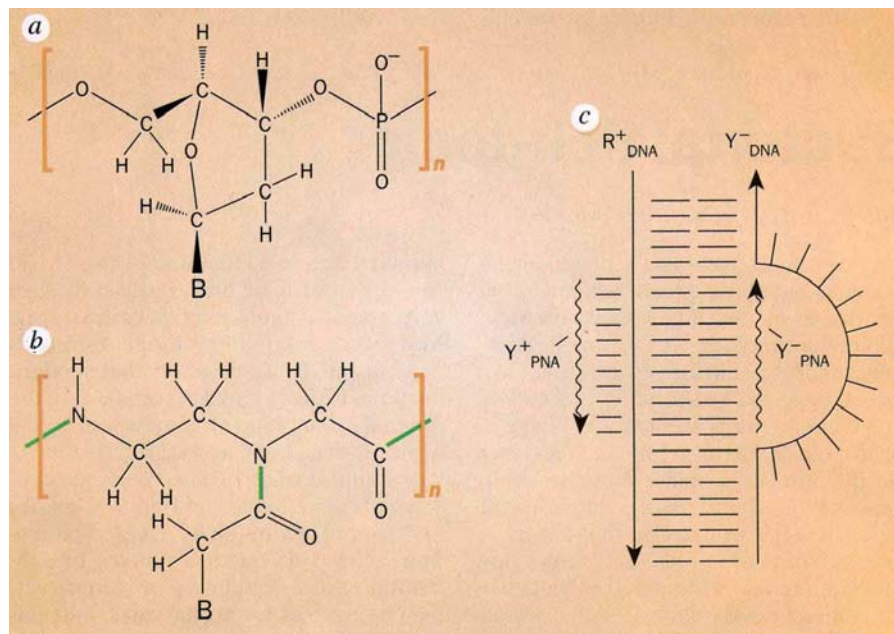
As to biological relevance, a few reports point to the potential for inhibition of certain processes through complex formation between homopyrimidine PNAs and their nucleic acid targets. These examples include complete inhibition of DNA cleavage by restriction enzymes when their binding sites span either edge of a bound homopyrimidine PNA complexation site¹⁰; this effect does not propagate along the helix, because no such inhibition was observed when the restriction site was moved five base pairs from the PNA complexation site. In another example, arrest of transcription elongation was observed at homopyrimidine PNA binding sites on the transcribed strand downstream from an RNA polymerase promoter site^{11,12}. Site-specific termination of both translation and reverse transcription has also been observed at PNA-RNA complexation sites¹². Finally, a gene-specific antisense effect has been reported for PNAs microinjected into mammalian cells and targeted to SV40 large T-antigen messenger RNA¹². In this study, the 15-mer PNA sequence contained a mixture of T, A and C residues and was one step removed from incorporation of all four natural bases in the PNA oligomer sequence.

The latest work with mixed-sequence oligonucleotide PNAs involved annealing studies with 15-mer PNAs, through which Egholm *et al.*¹ established formation of both PNA-DNA and PNA-RNA duplexes stabilized by Watson-Crick A·T and G·C pairing. The antiparallel orientation of strands was favoured in both PNA-DNA and PNA-RNA duplexes.

The increased thermal stability of antiparallel PNA-DNA duplexes over their DNA-DNA counterparts at low ionic strength must be associated with the lack of electrostatic repulsion between the uncharged PNA and the charged DNA strands, because both duplexes exhibit equal thermal stabilities at high ionic strength. There is a decrease in the thermal stability on introduction of single

before complex formation occurred. So there is also a need to evaluate the ability of mixed-sequence PNAs to destabilize RNA structural motifs in a manner analogous to potential strand invasion in duplex DNA.

A unique feature of PNAs is the marked stabilization of their complexes with nucleic acid target sites relative to that seen with oligonucleotide-based therapeutic



a, The DNA repeating unit, emphasizing the chiral centres. b, Achiral PNA repeating unit, with the peptide bonds in green. B marks the attached base. c, (PNA)₂-DNA triplex resulting from strand invasion and D-loop formation. R and Y stand for purine and pyrimidine, and + and - define the directionality of the strands. The Y⁺_{PNA} strand represents the third strand positioned in the major groove of the R⁺_{DNA}·Y⁻_{DNA} duplex.

mismatches in antiparallel PNA-DNA duplexes, the effect being more pronounced than in the corresponding DNA-DNA counterparts. Similar thermodynamic and kinetics parameters are observed for the duplex-strand transitions in antiparallel PNA-DNA and DNA-DNA duplexes. When coupled with the similar circular dichroism spectra for these duplexes, this result suggests that antiparallel PNA-DNA duplexes adopt base-stacking patterns that are similar to their DNA-DNA counterparts in solution.

A key distinction between homopyrimidine and mixed-sequence PNAs is that the former bind through (PNA)₂-DNA triplex formation whereas the latter form PNA-DNA duplexes. The unanswered question is whether mixed sequence PNAs can strand invade into duplex DNA through D-loop formation. If they can, this would be a way for targeting any sequence in the human chromosome. In addition, mixed-sequence PNAs should permit the targeting of RNA sequences through PNA-RNA complex formation. If the RNA target site is involved in higher-order folding, it would be necessary to destabilize or disrupt the fold

agents involving localized substitutions in the ribose-phosphate backbone. It is truly remarkable that the conformation and energetics of PNA-DNA complexes are so similar to those of their DNA-DNA counterparts and that the same holds true for RNA. In addition, the uncharged polyamide backbone of PNAs not only helps cell permeation and stability against nuclease cleavage, but also presents an achiral scaffold that can be synthetically modified in the future. Such modifications could include the introduction of

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chiral centres, and cation- and anion-binding sites, and substitutions that modulate PNA solubility. It is also conceivable that the polyamide backbone can be modified to generate PNAs that recognize left-handed rather than right-handed DNA, as well as tertiary interactions in RNA.

Further improvements in the biological potency of PNAs could result from conjugating them with ligands known to facilitate permeation into cells; indeed,

for the near future, one can envisage PNA analogues that are tailor-made to help tackle specific biological questions at the cellular level. But the main hopes for this technology are for drug therapies — success there will depend not only on efficacy, but on cost. □

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EVOLUTIONARY BIOLOGY

Bacterial tick-tock

Paul H. Harvey and Robert M. May

THE expanding catalogues of molecular sequence data have greatly extended our knowledge of the phylogenetic relations among prokaryotic organisms¹. It is, however, often difficult to assign a clear chronology to prokaryotic evolution; molecular methods are better at giving us the topology of phylogenetic trees than specific branch lengths. New work by Nancy Moran and others² focuses on various species of bacteria that are internal symbionts of aphids, and shows how information about the dated phylogenies of the insect hosts can be used to calibrate the molecular clocks of bacteria.

For multicellular plants and for animals — especially vertebrates — fossil evidence enables us to construct phylogenetic trees with fairly reliable dates on the nodes, and chronological lengths on the branches. Such chronologies can then be combined with molecular sequence data to estimate rates of base-pair substitution. Conversely, the substitution rates thus estimated can be used to assign tentative dates to phylogenetic events for which there is no fossil evidence. But, because prokaryotic fossils cannot usually be associated with modern organisms, there are few such direct calibrations of rates of molecular evolution among bacteria.

Boundaries

Ochman and Wilson³ have sought to infer the rates at which molecular clocks tick for bacteria, by using ecological arguments. They propose that large-scale ecological events, which can be roughly dated, can put upper and lower boundaries to the origins of bacterial lineages associated with particular habitats. For instance, those bacteria associated with the nodules of leguminous plants must have diverged from related bacterial taxa after the origin of land plants, but before the origin of legumes. Using palaeobotanical evidence, Ochman and Wilson date the common ancestor of the *Rhizobium* genus (in nodules) and the *Agrobacterium* (not in nodules) at between 415

million years and 110 million years (Myr) ago. Inferences of this kind clearly give very rough estimates of ancestral ages. Moreover, even these rough estimates depend on the assumption that modern bacterial taxa are lineal descendants of the original occupants of a new ecological niche, which is not necessarily true.

It would be nice to have more accurate ways of dating nodes and branch-lengths in bacterial evolutionary trees. For one thing, the consequent estimates of substitution rates would help in reconstructing phylogenies for prokaryotes, and elucidating the underlying mode and tempo of evolution^{4,5}. For another, such chronologies would allow us to test ideas about how generation times and other factors may affect substitution rates among bacteria, causing the molecular clock to tick faster along some branches than others^{6,7}.

Moran *et al.*² estimate such dates for a group of bacteria mutualistically associated with aphids. These endosymbiotic bacteria live within specialized aphid cells, are passed by maternal inheritance (vertical transmission) from aphid to aphid, and are essential for the growth and reproduction of their hosts⁸. Analysis of the 16S ribosomal DNA (rDNA) sequence has established that the bacteria are a monophyletic group or distinctive clade within the Proteobacteria⁹. Their phylogeny has a one-to-one correspondence with that of their diverse group of aphid host species^{10,11}. This precise concordance implies a long-term cospeciation of bacteria and aphids, resulting from vertical inheritance from an ancestral aphid in which the initial infection occurred.

There is a fairly good fossil record for the aphids, which allows their phylogenetic tree to be dated. The evidence for synchronous radiation of the aphid's endosymbionts thus enables consequent dates to be assigned to divergence events among the bacteria. Moran and colleagues' analyses of rDNA sequences show that rates of base substitution are

similar for all the different endosymbiotic lineages, and (from calibration against aphid dates) that they correspond to a roughly constant rate of 0.01 to 0.02 base substitutions per site per 50 Myr. These estimates of rates are amongst the best yet available for prokaryotes. The estimated range lies slightly above the earlier, and much rougher, estimate (0.01 substitutions per site per 50 Myr) made by Ochman and Wilson. Moran and colleagues' finding of approximate constancy in rates of base substitution is further supported by their observation that each of their 12 aphid endosymbiont species is distanced from *Escherichia coli* — one of the most closely related bacteria for which the 16S rDNA sequence is available — by about the same number of base substitutions (to within 10 per cent).

Divergence

Most of the aphid dates (10 of the 12 species) are based directly on fossils. The other two are melaphidine species (*Melaphis rhois* and *Schlectendalia chinensis*), one from America and the other from Asia. By using the roughly constant ticking of the rDNA molecular clock of the associated endosymbiotic bacteria, Moran *et al.* estimate that the American and Asian melaphidines diverged around 50–70 Myr ago. This is in cheering agreement with an earlier minimum estimate of 48 Myr ago, based on purely biogeographical considerations¹². Overall, this bacterial clock indicates that the minimum age of this mutualistic association is around 160–280 Myr. And this, in turn, is a good estimate of the age of the common ancestor of modern Aphidoidea, or the aphid Eve as it were.

More generally, the consistency between the findings of Moran *et al.* and the earlier estimates encourages us to believe that rates of 16S rDNA base substitution may be sufficiently constant, "even among organisms with very different lifestyles, that molecular clocks may be widely useful for reconstructing the evolutionary history of prokaryotes"². □

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Influence of the inner core

David Gubbins

THE dynamo theory for the origin of the Earth's magnetic field is notoriously difficult. The equations are basically those of physical oceanography and meteorology with the added complications of the magnetic field; laboratory experiments involve large masses of dangerous liquid metals that are difficult to instrument; numerical simulations are comparable in scale to those of weather forecasting; and observations are scarce.

Against this background, progress must be made through highly simplified numerical models and success measured in terms of a general understanding of the underlying physics rather than specific matching of theory to observation. On page 541 of this issue, Hollerbach and Jones¹ pull out a couple of surprises from a relatively simple model: incorporating a solid inner core, they find, stabilizes the dynamo and establishes a constant polarity such as we have on the Earth; furthermore, the magnetic field solution oscillates strongly at depth within the core but fluctuates only mildly at its surface. Perhaps we see only the tip of the iceberg in terms of magnetic field changes during times of stable magnetic polarity, and the mild fluctuations occasionally exceed a threshold that triggers a magnetic reversal.

The core occupies the central 3,500 km of the Earth, about half the total radius; the outer core is liquid iron, whose flow generates the magnetic field. Towards the Earth's centre, both pressure and temperature increase; the increase in pressure raises the melting point of iron. At a radius of 1,216 km the melting point reaches the ambient temperature, and the central inner core of the Earth is solid iron. Its cooling supplies energy for the dynamo, but the inner core's inductive effect on the magnetic field has always been considered negligible because it is small and buried deeply within the rest of the core. The new results therefore come as something of a surprise.

Core dynamics are strongly influenced by rotation, and the governing law is the Proudman–Taylor theorem: flow of a rapidly rotating fluid dominated by Coriolis forces cannot vary along the rotation axis. These conditions certainly hold in the Earth's core, although we are unsure how far the magnetic field removes the Coriolis constraint. Convection in rotating spheres takes the form of rolls aligned with the rotation axis, thus satisfying the Proudman–Taylor theorem except near the ends, where the slope of the spherical boundary makes it impossible. In the presence of an inner core the most unstable convection rolls touch the inner

core so that their ends are close to the 'flattest' part of the boundary². The sphere can be thought of as splitting neatly into two distinct regions, inside and outside a cylinder inscribing the inner core and with its axis parallel to the rotation axis. In polar regions, inside the cylinder, the fluid attempts to rise along the rotation axis in order to carry heat (or light material) outwards but is immediately in violation of the Proudman–Taylor theorem. Light fluid in low latitudes (outside the cylinder) rises, but its flow is turned to the form of rolls by the Coriolis force. On the Earth this cylinder has a radius about the poles of around 21° and there is some evidence that the magnetic field is influenced by it, with magnetic flux at the core surface concentrated away from the poles and around this inner core circle³.

Hollerbach and Jones's model makes a common assumption that magnetic field is generated through an ' α -effect'. The assumption, a drastic one, is that small-scale or non-axisymmetric flows act on the large scale to induce electric current parallel to the magnetic field — opposite to the macroscopic physical effect of an electrical conductor cutting magnetic field lines, which generates electric current perpendicular to the field. The underlying theory was developed independently for the Earth⁴ and the Sun⁵ and has formed the basis of most subsequent dynamo work. There are serious worries about its application to the Earth (the energy budget is inadequate and it predicts only axisymmetric magnetic fields, for example) but the model yields tractable equations that are probably a fair guide to the time evolution of the main dipole magnetic field and the macroscopic dynamics of the core, in particular its differential rotation and meridian circulation.

Meridian circulation seems to favour the generation of steady rather than oscillatory magnetic fields in kinematic dynamos, in which the fluid flow is prescribed rather than being obtained as a solution of the equations of motion⁶. The result appears to hold for full three-dimensional dynamos (D. G., manuscript in preparation), but it has proved exceedingly difficult to obtain a suitable form of meridian circulation from a dynamical model⁷, and most such dynamos generate oscillatory or chaotic magnetic fields. Why should the Earth's magnetic field be so stable?

Hollerbach and Jones find that the inner core imparts that stability. First, dynamo action is restricted mainly to the region just outside the inner-core cylinder, with magnetic flux expelled from this

entire cylinder. This, it seems, favours steady fields by generating the appropriate meridian circulation. Second, the electrical diffusion time of the inner core lengthens the timescale for change in the magnetic field and essentially averages out any wild fluctuations in the magnetic field that might lead to rapid reversal of the whole field. The result is a quasi-periodic solution that oscillates about a non-zero average.

A further remarkable property of Hollerbach and Jones's solution is the dramatic oscillatory changes deep in the core coupled with the rather mild variations in dipole moment. It suggests that the oscillations seen at present in the geomagnetic dipole, with a typical period of thousands of years⁸, may be a symptom of much greater magnetic changes deep in the core. The authors suggest that particularly large oscillations may develop into full reversals, thus developing a train of speculation that began with Cox's⁹ original phenomenological reversal model and continuing with the suggestion that the oscillations may be directly related to the flux expulsion going on at present beneath the South Atlantic¹⁰. Alas, the α -dynamo model is incapable of predicting such non-axisymmetric fields, but the new model is a big step forward in our understanding of how such a process might work.

It is an intriguing thought that the inner core can control magnetic reversals. Perhaps we should look towards a changing inner-core radius to explain the long-term change (over tens of millions of years) in reversal frequency, and the almost total absence of reversals for long intervals in the Cretaceous and Permian periods. These changes are normally attributed to changing conditions at the interface between core and mantle, but the inner-core radius will also vary on this timescale: if the core were colder at some point in the past, the melting point of iron would be reached further out and the radius of the inner core would be correspondingly larger, the condition favouring stable polarity in Hollerbach and Jones's model. □

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Renaissance of Europe's ape

Lawrence Martin and Peter Andrews

Dryopithecus was the first large fossil ape to be discovered but has lingered in obscurity for much of its history. Now it is back in the limelight. On page 543 of this issue¹, Moyà Solà and Köhler describe a new cranial specimen from the middle Miocene of Spain (about 10–12 million years ago) and claim to demonstrate that *Dryopithecus* is not only a member of the group comprising great apes and humans, but is particularly affiliated with the orang utan (*Pongo*). Begun² has recently argued that *Dryopithecus* is a close relative of the African apes. These contradictory views raise the question of what we really know about this European ape.

Dryopithecus fontani, the type species of the genus from southern France, was described by Lartet in 1856. In the early part of this century several new species were described from the middle and late Miocene of Indo-Pakistan, and subsequently material from Spain and Czechoslovakia was referred to one or other of the *Dryopithecus* species as well. *Dryopithecus* had thus become established as the default genus into which to put a fossil ape. The 1930s began to see a change from that position in the systematic revision of Lewis³, who limited the recognition of *Dryopithecus* to Europe. New Miocene fossil apes from China were later described as belonging to the genus, but the basis for this assessment was their resemblance to specimens from Czechoslovakia that are more often considered to belong to *Sivapithecus*, or more recently to *Griphopithecus*.

The main revision of Miocene apes in the modern period was that of Simons and Pilbeam⁴, who brought order into chaos by subsuming virtually all genera of Miocene large apes into *Dryopithecus*. But during the past 25 years, with major new discoveries of *Proconsul* and *Sivapithecus*, all workers in the field have come to accept that the taxonomic diversity of Miocene apes greatly exceeds what was recognized by Simons and Pilbeam.

Meanwhile, a steady stream of finds in Germany, Spain and Austria had added to our knowledge of *Dryopithecus*. In the late 1960s and early 1970s two principal collections emerged, the first from a number of localities in northeastern Spain and the second from Rudabánya in Hungary. In the past two years, *Dryopithecus* has leapt to prominence in the study of human origins, partly as a consequence of the recovery of important new cranial specimens from both countries. Begun² based his analysis on the Hungarian material, confirming the long-held view that *Dryopithecus* belongs to the great ape/

human clade. Subsequently, he has developed his argument that *Dryopithecus* is in fact specifically related to the African ape and human part of this clade⁵.

Moyà Solà and Köhler now provide additional evidence that *Dryopithecus* is indeed more closely related to great apes and humans than are the extant gibbons, based on their analysis of the subarcuate fossa. They take their analysis a step further and identify two characters of the zygomatic (cheek) region of the face that they interpret as shared derived between *Dryopithecus* and the orang utan. This in turn would imply that *Dryopithecus*, *Sivapithecus* and *Pongo* form a clade, within the great ape and human clade, that is no longer exclusively Asian in distribution.

Begun, and Moyà Solà and Köhler, cannot both be right. But both conclusions are based on very limited evidence, and neither analysis has settled the problem. For *Dryopithecus* to be either a relative of orang utans or a member of the African ape and human group (that is, to belong within the great ape and human clade), it must first be shown to possess the great ape and human clade synapomorphies for most known characters. Our analyses did not find this to be the case — like Begun, and Moyà Solà and Köhler, we found *Dryopithecus* to retain the ancestral condition for many characters for which great apes and humans share the derived state. Nonetheless, we concur with Begun and Moyà Solà and Köhler that *Dryopithecus* shares some derived characters with great apes and humans that warrant its recognition as the sister group of the great ape and human clade.

With the description and analysis of a second good facial specimen of *Dryopithecus*, this genus becomes one of the best known apes from the Miocene. There will undoubtedly be continuing controversy over its affinities. But whatever the final outcome, the new specimens and recent analyses of the morphology of *Dryopithecus* have demonstrated the critical importance of the only European Miocene ape for understanding hominoid phylogeny and human origins. □

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In from the cold

HOWEVER you go about it, slimming is a painful, almost masochistic endeavour. You must either take in fewer calories, or expend more of them. The second option is usually achieved by exercise, but exposure to cold should also work. This chilly alternative may seem almost equally unpleasant; but Daedalus has a plan to make it nearly painless.

Arctic explorers, shipwrecked mariners on wave-swept rafts, and snow-bound travellers, should all know one crucial rule for survival: no matter how cold you get, don't go to sleep! In sleep, the body relaxes its defences against cold. It loses heat so fast that its energy reserves rapidly run out; the sleeper may die quite quickly. So, says Daedalus, why not exploit this ready somnolent loss of calories? DREADCO engineers have built a prototype 'Frigibed' which freezes the sleeper, draining him of calories safely and controllably.

The occupant of the Frigibed wears a special nightcap. It carries an array of scalp-contact electrodes which record his skin temperature, and the EEG and REM signals from his brain and eye muscles. It transmits them by a local radio-link to a small computer, which deduces his depth of sleep, and adjusts the heaters and refrigeration units of the Frigibed accordingly.

While the occupant is awake, the Frigibed remains comfortably warm. As soon as he dozes off, it starts to cool down. As he plumbs the depths of sleep, the Frigibed plumbs the depths of temperature. His undefended body pours out calories to maintain its normal warmth. If the scalp-temperature or EEG start to falter ominously, the computer warms the bed up slightly; it mustn't freeze the sleeper to death. As he drifts towards wakefulness, the bed warms up more strongly. When he finally wakes up, he will find the Frigibed as warm and pleasant as when he went to sleep. During the night, knowing nothing about it, he will have lost hundreds of calories!

Intrepid DREADCO volunteers are now testing the prototype. Their main complaint is a tendency to dream of snowstorms and refrigerators. A sleeper waking suddenly in the night will find to his horror that such dreams have apparently come true. To avoid such frigid nocturnal surprises, Daedalus may have to fit the Frigibed with a powerful microwave flash-heater, to be triggered by signals of sudden waking. It will then restore a comfortable temperature instantly if the sleeper is startled into wakefulness. Truly masochistic slimmers, of course, may prefer to suffer its icy grip in full consciousness.

David Jones

Locomotion like a wheel?

SIR — Since the time of Sumer, our conception of efficient movement has been shaped by the wheel. Vehicles using wheels are efficient because they result in smooth rides. Wheel-like rides are possible even when legs are used for locomotion. Engineers, realizing the advantages of legged locomotion, have built many-legged robots that also minimize repeated accelerations and decelerations. Despite the efficiency of wheel-like motion, animals do not have wheels, possibly because the formation of a wheel-like structure is biologically problematic¹ and wheels are not necessarily advantageous on all surfaces^{2,3}.

The quest for efficient, wheel-like dynamics in legged robots led us to ask whether any animal in an appropriate environment actively moves like a wheel using its legs or body. Hoop snakes that catch their tail in their mouth to roll better may be mythical⁴, but desert spiders can pull in their legs and roll passively down a slope to escape danger⁵. Some coelenterates can cartwheel or roll by alternate attachment of the pedal disk and tentacles⁶. From a handful of candidates such as these, we thought that the most likely species to use active, wheel-like motion was a crustacean reported to roll along the beach⁷.

The small stomatopod *Nannosquilla decemspinosa* is found on the sand beaches of the Pacific coast of Panama. We

videotaped the somersaulting of this shrimp-like crustacean to see if it moved like a true wheel (Fig. 1). Because of their elongated body and short, laterally projecting legs, adults cannot walk when out of the water. When washed from their burrows and cast up on the beach by waves, they move across the sand by

in synchrony that also function as a single, effective 'leg'⁸. Animals seldom use wheel-like locomotion even when it is possible, and instead rely on pendulum or spring-like mechanisms that require repeated oscillations of their bodies. Because most legged robots are dynamically more similar to turtles than faster and more manoeuvrable animals, perhaps future robotic designs can benefit from the transfer of biologically inspired ideas

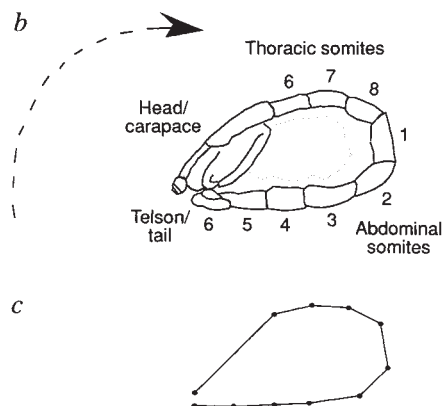


FIG. 1 *a*, Backwards somersault of a stomatopod. *b*, The body of *N. decemspinosa* consists of a head/shell, thorax, abdomen and tail. *c*, Spatial model of a stomatopod used for motion study.

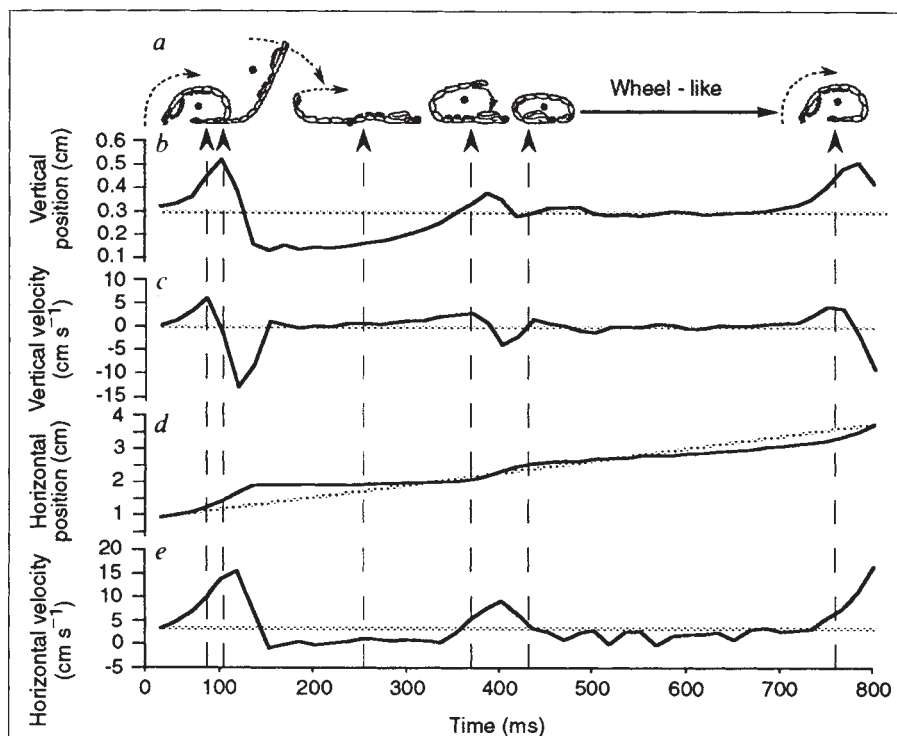


FIG. 2 Motion of the stomatopod's centre of mass (filled circle) during one backwards somersault. *a*, The stomatopod uses its tail as a single leg to lift its body up and move it forwards. After a slight pause on its back, the stomatopod lifts its tail and curls it up over the body. The body then acts as a leg to push the tail section up and over. When the tail touches the ground the stomatopod rolls like a true wheel with the centre of mass at the hub. *b*, vertical position; *c*, vertical velocity; *d*, horizontal position; *e*, horizontal velocity.

backwards somersaults for as far as 2 m, completing as many as 20–40 consecutive rolls⁷. Stomatopods actively propelled themselves forwards at 72 revolutions per min or 1.5 body lengths per s (3.5 cm per s) and rolled as a true wheel for 40 per cent of each revolution (Fig. 2). Sixty per cent of a cycle was marked by large accelerations and decelerations similar to the patterns generated by legged walkers and runners. When the animal could not capitalize on wheel-like motion, its body functioned as a single leg to thrust itself upwards and forwards.

The unique stomatopod somersaulter accelerated and decelerated its body each cycle by using a single, effective 'leg', as do many-legged animals who also had been presumed to move like wheels (supposedly, the more legs operating in a wave, the more wheel-like is the movement). Instead, insects, crabs and centipedes tip or bounce by using several legs

that represent alternatives to wheel-like motion⁸. Who knows how many of these alternatives are waiting to be discovered in nature?

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Fatty acylated toxin structure

SIR — The ω -conotoxins^{1,2}, and a variety of spider toxins^{3–11}, have been essential to our emerging understanding of voltage-dependent Ca^{2+} channels. Toxins from the venom of *Plectreus tristes* (PLTX)^{3,4} were among the first presynaptic calcium-

carboxy terminus (Fig. 1). In proteins, palmitic acid has typically been found in thioester linkage to cysteine residues¹², although an *O*-palmitoyl linkage has been shown in myelin lipophilin¹³. PLTX-II is the first example of a C-terminal, *O*-linked palmitoyl moiety, but the structure bears obvious similarity to functionally important *S*-palmitoyl and *S*-isoprenyl modifications associated with a range of regulatory proteins, including many G proteins^{12,14–17}.

Although they are primarily water-soluble peptides, *Plectreus* toxins have certain physical properties such as significant solubility in organic solvents and strong retention on reversed-phase high performance liquid chromatography (RP-HPLC)³ which imply a hydrophobic

structural component. The mass of native PLTX-II determined by ion spray mass spectrometry is $5,108.8 \pm 1.3$ AMU. Amino acids 1–42 account for about 90 per cent of the mass, the rest being in a small, hydrophobic tryptic fragment beginning with Cys 42 (680.4 AMU). Amino-acid analysis suggests that the C-terminal sequence is

Cys-Asp-Thr, but this sequence accounts neither for the entire mass nor for the lipophilic properties. The collision-induced dissociation (CID) mass spectrum of the fragment suggests that the C-terminal Thr is carboxyl-amidated and *O*-palmitoylated (Fig. 2a,b) and the hydrolysate of the fragment contains palmitic acid in approximately equal ratio with Thr. We therefore synthesized the *S*-pyridinethylethylated, *O*-palmitoyl compound (Fig. 2a)¹⁸. The synthetic and native compounds both have the predicted mass of 680.4 AMU, eluted at 53 per cent acetonitrile in gradient RP-HPLC, and produce identical CID-mass spectrometry patterns (Fig. 2b,c). The average mass of the proposed structure for the entire toxin with five disulphides is 5,109.8 AMU.

Fatty acylation seems to be a property of all similar calcium-channel blockers in *Plectreus* venom. Cleavage of the *O*-palmitoyl ester from native toxin by base treatment results, as expected, in a decreased RP-HPLC retention and a loss of biological activity (a drop in potency of at least two orders of magnitude), suggesting that fatty acylation is required for activity. Post-translational addition of lipid to proteins is known to alter function. Lipids can target hydrophilic proteins to

Ala Asp Cys Ser Ala Thr Gly Asp Thr Cys Asp His
Thr Lys Lys Cys Cys Asp Asp Cys Tyr Thr Cys Arg
Cys Gly Thr Pro Trp Gly Ala Asn Cys Arg Cys Asp
Tyr Tyr Lys Ala Arg Cys Asp Thr(Pal)amide

FIG. 1 Structure of PLTX-II. Toxin was purified from *P. tristes* venom as previously described^{3,4}. A detailed description of the sequence determination will be presented elsewhere. The C-terminal tryptic fragment analysed in detail in Fig. 2 is underlined. Pal, *O*-palmitoyl linkage.

channel blockers to be isolated from spider venom, but their chemical structures have been elusive until now. We have recently determined the complete structure of one member of this family, the insect toxin PLTX-II, which is a 44-amino-acid peptide with an *O*-palmitoyl threonine amide residue at its

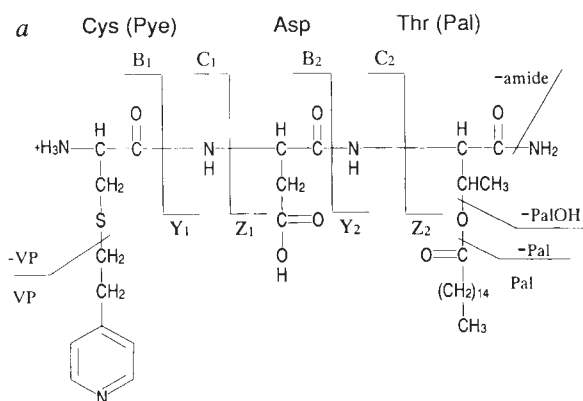
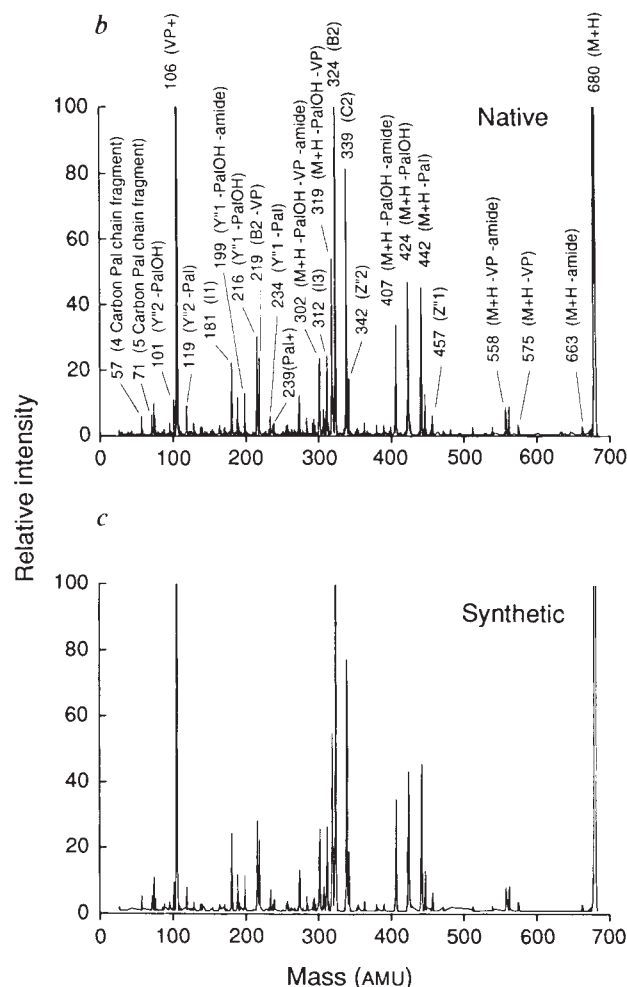


FIG. 2 Analysis of the HPLC-purified, C-terminal tryptic fragment of pyridinethylethylated PLTX-II by mass spectrometry using a Sciex API III tandem mass spectrometer with an ion spray interface. a, Proposed structure with key points of dissociation labelled. Standard B-, C-, Y- and Z-type fragments around the peptide bond are according to ref. 20. The molecular ion (M+H) and some standard fragments can also lose characteristic subfragments and retain a positive charge. The carboxylamide is lost as NH_3 (–17 AMU); the pyridinethyl protecting group of Cys can be lost as vinyl pyridine (–105 AMU); and the palmitoyl moiety can be lost with (–256 AMU) or without (–238 AMU) concomitant loss of H_2O from the side chain of Thr. b, CID spectrum of the native C-terminal fragment M+H (680 AMU). Nominal mass (AMU) and proposed structural assignments are given for key fragments using the terminology in a. I_1 and I_3 are internal fragments representing Cys(Pye) and Thr(Pal), respectively. c, CID spectrum of synthetic Cys(Pye)-Asp-Thr(Pal)amide is identical to the spectrum of the native material, confirming identity of the compounds. (None of the ions uniquely assigned as Pal fragments was present in CID spectra generated from Cys(Pye)-Asp-Thr-amide.)



membranes, and alter protein-protein interactions^{12,14-17}. The *O*-palmitoyl structure described here may have specific significance for the action of this class of toxins. It is even conceivable that the lipid moiety allows toxin to penetrate membranes and act at an intracellular site¹⁹. The specific molecular mechanism of action of PLTX-II is not yet known, but components of *Plectreureys* venom are allosteric, noncompetitive inhibitors of ω -conotoxin binding in vertebrate brain⁵.

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Scientific Correspondence

Scientific Correspondence is a relatively informal section of *Nature* in which matters of general scientific interest, not necessarily those arising from papers appearing in *Nature*, are published. Because there is space to print only a small proportion of the letters received, priority is usually given according to general interest and topicality, to contributions of fewer than 500 words, and to contributions using simple language.

Perception of heading

SIR — Royden *et al.*¹ demonstrate that people can accurately perceive their direction of heading across the ground plane while they pursue a moving object with their eyes. When the same retinal motion is presented to stationary eyes, large errors in perceived heading occur. The latter result is in conflict with earlier findings^{2,3} that the heading percept is equally accurate in both conditions. Royden *et al.* conclude that the different outcome is caused by their use of 2–5 times faster simulated eye rotations as compared to those in ref. 2. If true, the visual stimulus alone is not sufficient for heading perception during natural locomotion; extra-retinal signals are required to perceive heading when making eye movements.

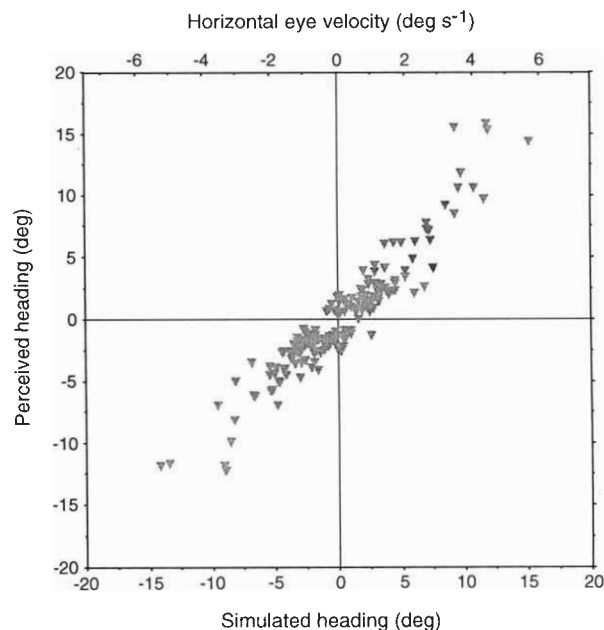
In the earlier studies^{2,3}, the fixation point was part of the rigid environment rather than an independently moving object. To exclude this difference as the cause for the conflict, Royden *et al.* demonstrated that subjects also could not perceive the direction of heading when they had presented to their stationary eye the retinal flow during forward motion through a rigid cloud of points, of which one was fixated. This demonstration is flawed because it confounds the effects of the layout of the points (planar in the main experiment and a cloud in the control experiment) and the motion of the fixation point. People do accurately perceive their direction of heading when fixating a point in the ground plane, even when the average simulated eye rotation is 5° s^{-1} (ref. 3) (see figure). This rotation rate is close

to the maximum eye rotation used by Royden *et al.* In agreement with their results, heading perception during simulated eye rotation was grossly inaccurate when motion through a cloud of points was presented³.

Contrary to Royden *et al.*, I believe that the visual stimulus by itself is generally sufficient for perception of heading when making eye movements during locomotion,

provided that the fixated target is part of the rigid environment. Current models for heading perception that visually decompose the retinal flow into a component due to the rotation and a component due to the translation of the observer would not predict such a requirement for the fixated object. In this respect, the new result¹ questions the validity of these models.

Although extra-retinal signals may not be required for heading perception in



Perceived heading of one observer for motion across a plane of 256 randomly positioned white points. Forward motion of the observer (3 m s^{-1}) and the eye rotation required to fixate one red point in the plane were simulated in the display. This point was presented at the centre of the screen (dimensions: 60° horizontally; 50° vertically) and fixated by the subject. Each trial started with presentation for one second of the stationary scene followed by 0.83 s of motion. The simulated distance of the fixation point was initially 8 m . Initial eccentricity of the heading direction was varied between 0.25 and 10° . Subsequently, a probe appeared that the subject could move in the plane of dots along a circle through the fixation point that was concentric with the subject's feet. The subject pointed with the probe in the direction of heading. Indicated in the figure are the perceived and the simulated heading directions at the end of the motion sequence relative to the fixation direction. The direction of heading was perceived accurately up to about 15° eccentricity. As indicated in the upper scale the average horizontal eye rotation may exceed 5° s^{-1} . Although the scatter in the perceived heading increases for larger eccentricity, there is no indication that the perceived heading was biased towards the fixation point. The threshold for heading discrimination as estimated from the scatter would vary between 1 and 2.4° , depending on the average eye rotation.

many cases, there is evidence that these signals can contribute to the heading percept. Information concerning heading is degraded for motion towards a fronto-parallel plane and when many noisily

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moving objects are in view³. Heading perception is much more robust for adverse conditions such as these^{2,3}, when the subject makes real rather than simulated eye movements.

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Time for tea

SIR — M. Spiro and D. Jaganyi (*Nature* **364**, 581; 1993) suggest that a chemical reaction takes place between tea and the suspension of calcium carbonate present in boiled hard water. I believe, however, that the main process taking place is physical adsorption of the coloured tannin from the tea on to the surface of the fine suspension of calcium carbonate that then settles on the surfaces of teapots and cups as a brown stain. Any chemical reaction is negligible. It is common practice for tea drinkers to add citric acid in the form of a slice of lemon to remove the calcium carbonate and prevent the formation of a stain.

I have extended the process by adding a small quantity of citric acid crystals to the hard water before boiling. The calcium bicarbonate hardness is converted into soluble citrate and no precipitate is produced after boiling.

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Velocities in the mantle

SIR — The Scientific Correspondence¹ from Wright quoted my News and Views article² out of context, and its complaints were unwarranted. My article, about the recent results of Vidale and Benz³, explained that while regions of anomalously fast seismic compressional (P) and shear (S) velocities have been found in many places at the base of the mantle with many different data sources, the fast P-velocity core-mantle boundary feature found by Vidale and Benz was in a region (beneath Alaska) that had previously revealed slow P velocities⁴⁻⁶. Material exhibiting fast P-wave velocities certainly exists at the base of the mantle, but it was not expected in this region. The statement that led to confusion, "The results are all the more intriguing because they do not occur with previous studies", was therefore meant in a regional and not a global context.

Wright interprets this sentence as an

implication that no other regions of fast seismic velocities had ever been found, says that my statement is incorrect, and cites some examples of his own work in which he found fast seismic zones at the base of the mantle in other parts of the world. His comments are unjustified, because the rest of the paragraph in my News and Views that contains the above quote lists nine other studies within the past 6 years in which fast seismic zones at the base of the mantle are reported. I am well aware, and made it clear, that the base of the mantle can display high-velocity zones. I certainly recognize the contributions that Wright has made to this subject, but point out that News and Views articles are intended to put pieces of current research into context. They are not designed to give a thorough historical background to the subject, nor could this be done with a dozen or fewer references.

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OH in Saturn's rings

SIR — The presence of the hydroxyl radical (OH) near the inner satellites of Saturn is not unexpected as the adjacent moons and rings are predominantly water ice. Nevertheless, the discovery^{1,2} of OH is surprising because the amount observed implies a production rate of the H₂O parent molecule 20 times greater than theoretical calculations would suggest¹. Typical sources for circumplanetary H₂O include collisions of interplanetary micrometeoroids into inner satellites and rings, and sputtering of these objects by ions and neutral particles. To account for their observations, Shemansky *et al.*¹ suggest that the interplanetary micrometeoroid flux to the saturnian system could be 20 times greater than currently believed. But such an increased flux would dramatically shorten lifetimes for the main saturnian rings to darken, for the rings to spread by angular momentum transfer and for ring particles to erode, which would make theories of the rings' primordial origin untenable. Instead, we suggest that H₂O is primarily produced by collisions of E-ring grains into the inner satellites of Saturn.

The faint E ring is composed of

micrometre-sized icy debris, which is thought to be chips off the inner saturnian satellites^{3,4}. The orbits of these tiny grains are strongly perturbed by the non-gravitational forces of electromagnetism and radiation pressure, which cause initially circular orbits periodically to become highly elliptical³. These elongate paths bring E-ring grains across the nearly circular orbits of the inner satellites so that an average E-ring grain will strike one of the moons with a typical velocity of about 2.5 km s⁻¹ in about 20 years. These hypervelocity impacts eject relatively large amounts of vapour and sub-micrometre-sized debris into orbit around Saturn.

We now compare the relative importance of the E ring and interplanetary micrometeoroid contributions to the production of H₂O. Using values from refs 5 and 6, the total mass fluxes to the satellite Enceladus are estimated as follows:

$$\begin{aligned} \text{Interplanetary micrometeoroid flux:} \\ (\text{IP flux density}) (\text{area of Enceladus}) = \\ (4.5 \times 10^{-17} \text{ g cm}^{-2} \text{ s}^{-1}) \pi (250 \text{ km})^2 = \\ 0.088 \text{ g s}^{-1} \end{aligned}$$

$$\begin{aligned} \text{E-ring particle flux:} \\ (\text{mass of E ring}) / \\ (\text{collisional timescale with Enceladus}) = \\ (7 \times 10^{11} \text{ g}) / (20 \text{ yr}) = 1,100 \text{ g s}^{-1} \end{aligned}$$

it is seen that the flux of E-ring grains to Enceladus exceeds the nominal flux of interplanetary micrometeoroids by a factor of about 10,000. Even after accounting for the larger collision speeds of the interplanetary particles (about 25 km s⁻¹), we predict that the E-ring grains will produce collisional products at least 100 times more efficiently than their interplanetary counterparts.

Mutual collisions among E-ring particles also occur relatively frequently and at high velocity. These grain-grain collisions further enhance the efficiency at which micrometre and sub-micrometre-sized collisional products are converted into H₂O vapour. Incorporating E-ring particles into gas production calculations, we find that the amount of material blasted from the satellites is sufficient to account for the missing factor of 20 in the water production rates.

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Molecular tectonics in biomineralization and biomimetic materials chemistry

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The systematic fabrication of advanced materials will require the construction of architectures over scales ranging from the molecular to the macroscopic. The basic constructional processes of biomineralization—supramolecular pre-organization, interfacial molecular recognition (templating) and cellular processing—can provide useful archetypes for molecular-scale building, or ‘molecular tectonics’, in inorganic materials chemistry.

HIDDEN in the current stream of chemical publications is mounting evidence of a quiet revolution. Although most chemists remain firmly encamped within the classical divisions of inorganic, organic and physical chemistry, there is a growing number who are intent on exploring new frontiers in the apparent wilderness between established disciplines. One reason for this is an awareness of the diminishing returns to be extracted from synthetic chemistry that is based solely on the covalent bond. In its place is a chemistry that explores the relatively weak non-covalent interactions that can exist between molecules. When these intermolecular bonds act in a cooperative, convergent way, organized supramolecular systems can be constructed by self-assembly. A parallel can be drawn with biological aggregates such as DNA, in which hydrogen bonding and π -stacking interactions are important factors in governing molecular architecture.

Alongside this new pattern of molecular chemistry is the growing realization in inorganic materials chemistry that the traditional methods of ‘heat-and-beat’ will not be able to address the requirements of future advanced materials. Instead, an approach to materials synthesis based on molecular design and organized assembly is needed. Thus, while synthetic chemists are beginning to build beyond the molecule, materials chemists are developing skills in molecular engineering. Both perspectives are related in striving towards the chemical construction of higher-order architectures, albeit on different length scales—a process that I will define as ‘molecular tectonics’ (from the Greek *tekton*, builder).

The ability to construct organized nanoscale, microscopic and bulk inorganic materials from molecular components is of importance in electronics, catalysis, magnetism, sensory devices and mechanical design. In particular, the current thrust towards ‘intelligent materials’ relies on the interplay between structure, organization and dynamics in determining functional responses. Where better to look for archetypes of these systems than in the interactive, homeostatic milieu of biology? Much of the activity in intelligent materials is centred around the biomimicking of tissues such as muscle fibre and skin. But the ‘soft organic’ technology of chemo- and electro-active polymer gels is incompatible with the ‘hard and stiff’ approach of advanced materials engineering and robotics. No such demarcation exists in biology, as both inorganic and organic materials can be assimilated within responsive and adaptive biomineralized structures such as bone.

The study of biomineralization offers valuable insights into the scope and nature of materials chemistry at the inorganic–organic interface¹. Biological systems are replete with examples of organic supramolecular assemblies (double and triple helices, multisubunit proteins, membrane-bound reaction centres, vesicles, tubules, and so on), some of which (collagen, cellulose, and chitin) extend to microscopic dimensions in the form of hierarchical structures². Here, the interest is in how these organic architectures can be associated with inorganic solids to give unique and exquisite biominerals (for example, diatom frustules, coccoliths, seashells, bone, and so on) in which the structure,

size, shape, orientation, texture and assembly of the mineral constituents are precisely controlled³. For an increasing number of scientists, these bioinorganic materials represent a source of inspiration for materials synthesis. What captures the imagination is how such relatively simple inorganic minerals (CaCO_3 , SiO_2 , Fe_3O_4 , and so on) can be formed into precise functional architectures. For example, tough, durable and adaptive polymer–ceramic composites can be fabricated using calcium phosphate or calcium carbonate by organized assembly based on specific molecular interactions. Magnetic, gravity and storage devices can be similarly constructed¹. If these biological archetypes can be reformulated in a synthetic context then perhaps the biomimetic design of nano- and microscale materials and composites based on inorganic materials could be a real possibility in future processing strategies.

In this article I propose that the study of the molecular processes that result in the construction of higher-order architectures in biomineralization is relevant to the development of new strategies for the controlled synthesis of organized inorganic and composite materials. There are other strategies to similar ends, for example involving chemical vapour deposition, which are actively being explored⁴⁹. My intention is not to provide a critical review (see refs 1 and 3) but to present a commentary, using representative examples, on the essential aspects of molecular tectonics that connect materials chemistry and biomineralization, and ultimately their assimilation within a biomimetic approach.

Molecular tectonics and materials chemistry

A number of chemical strategies are now available for the construction of higher-order structures. Organic molecules can be linked together by molecular recognition. For example, synergistic non-covalent donor–acceptor interactions can give rise to intertwined rings (catenanes)⁴. Liquid-crystal polymers having self-organized structures can be formed from organic molecules containing headgroups capable of complementary hydrogen-bonding interactions⁵. Alternatively, organic molecules can be assembled around metal ions such as Cu(I) which provide stereochemical constraints in the construction of double helices⁶. The synthesis of inorganic clusters, by contrast, is usually dependent on passivating the surface of a growing aggregate by capping the surface sites with stabilizing ligands. For example, compound clusters containing Fe–O bonds are stabilized by carboxylate and alkoxide ligands^{7,8}, Ni(Cu)/Se aggregates are stabilized by phosphine groups^{9,10}, and CdS clusters are stabilized by phenylate ligands¹¹. Metal clusters such as Pd capped with phenanthroline groups¹², Au with phosphine ligands¹², and Pt(Ni) with carbonyl ligands¹³ have been studied. Unfortunately, the size and structure of these nanoscale particles often remain unpredictable because there is little information about the interplay between surface (ligand) and internal (lattice) energies during cluster growth. In some systems, the structures are distorted by the stereochemical constraints of the capping ligands, whereas in

other compounds, the clusters resemble crystalline fragments of a corresponding bulk phase.

How large can we build by these methods? At present, most compound clusters contain less than 100 atoms. One possibility might be to increase the spatial extension of the ligand set at the growing cluster surface by using polyfunctionalized molecules (for example, peptides and oligomers) that contain tandem repeats of binding sites. For example, whereas most metalloproteins can accommodate only small metal-ion clusters (metallothioneins can bind up to 18 metal (Hg) ions¹⁴), a series of short-chain glutathione-like peptides containing the repeat motif (γ -GluCys)_{*n*}Gly (*n* = 1–3) is used to stabilize the intracellular construction of crystalline CdS clusters in yeast cells¹⁵. These biogenic semiconductor particles contain ~85 metal ions and are capped by the cysteinyl thiolate residues of the adsorbed peptides. A similar strategy has evolved in the stabilization of amorphous calcium phosphate clusters in milk proteins, except that the ligand set consists of blocks of phosphoserine residues¹⁶.

An alternative approach to the construction of inorganic clusters is based on host–guest chemistry. Inorganic materials such as zeolites can house a variety of inorganic guests within their pores. This confined space (up to 1.2 nm across) can be employed as a reaction environment for the production of small clusters, such as Ag¹⁷, CdS¹⁸ and GaP¹⁹. Similarly, laponite and montmorillonite clays have been used as hosts for the intercalation of CdS²⁰ and Cu²¹ clusters, respectively, and Zr phosphonate has been employed in the synthesis of ZnSe and PbS²². Recently, carbon nanotubes have been used as unidimensional reaction environments for the trapping of LaC₂²³ or metallic Pb²⁴. Whereas these approaches use the nanoscale porosity of preformed hosts to assemble cluster materials, the converse—the use of small-scale aggregates to promote the assembly of mesoporous microstructures—has long been a major strategy in the chemistry of zeolites and zeolite-like materials. A wide range of organic ‘templates’ have been used; historically these have been based on quaternary ammonium salts, although recent work has involved self-assembled surfactant aggregates²⁵. The latter have been used to produce, for example, aluminosilicate materials, with pore diameters up to 10 nm, by inducing the nucleation and growth of the inorganic material within the interstices of a preformed hexagonally packed liquid-crystal assembly of cylindrical surfactant micelles²⁶.

In summary, strategies involving molecular recognition, ligand capping, host–guest cluster chemistry and molecular templating are being explored in the construction of higher-order architectures in materials chemistry. In the next section, I will show how similar processes operate in biomineralization (except for ligand capping, which is relatively uncommon (see refs 15 and 16 for examples)). It should be pointed out that the tectonic capability of these biological systems far exceeds that currently realized in synthetic chemistry, because of the cellular processing of organized architectures.

Molecular tectonics in biomineralization

The old adage that life is merely advanced aqueous organic chemistry has been superseded for the most part by a view that embraces the interconnectivity of metal and non-metal elements in the evolution of biochemical processes²⁷. For the most part, the study of bioinorganic chemistry has been synonymous with biocoordination chemistry, and it is only recently that length scales beyond that of metal–ligand macromolecular systems have been considered important. In the context of materials chemistry, both bionanochemistry and bio-solid-state chemistry could be invaluable sources of knowledge and inspiration. Here I focus on biomineralization which encompasses aspects of both these fields, although they are distinguished by differences in their dimensionality and constructional complexity.

Nanoscale biomineralization involves the molecular construction of discrete self-assembled organic supramolecular systems (ferritin, vesicles, and so on) that are used as pre-organized

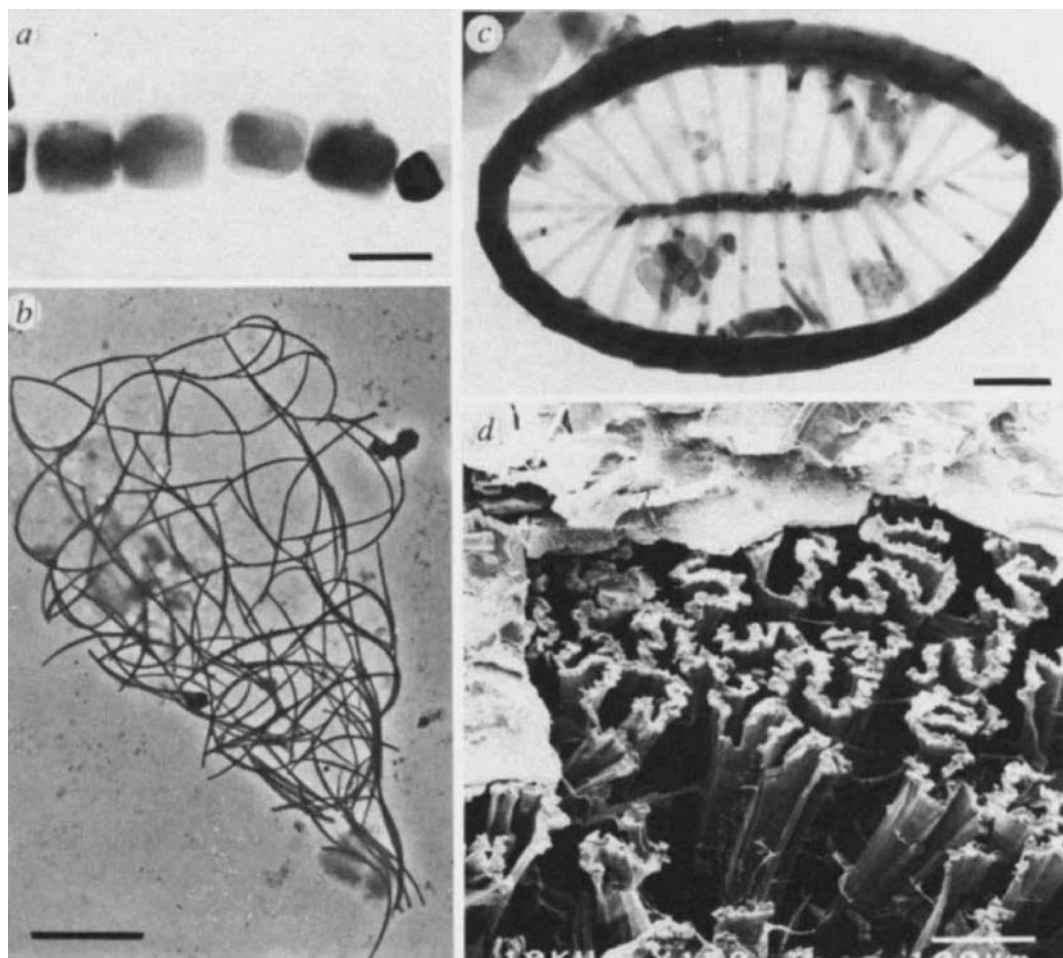
environments for controlling the formation of finely divided inorganic materials, 1–100 nm in size¹. The fabrication of consolidated biominerals, such as bone and teeth, also involves the construction of pre-organized organic frameworks, but the length scale is greater (micrometres) and the matrix is often polymeric (such as collagen and enamel tubules)³. The construction and use of discrete and extended organic architectures in biomineralization parallel the strategies employed in materials chemistry because both involve processes of molecular recognition, host–guest chemistry (pre-organization) and molecular templating. One profound difference, however, is that biomineralized structures can be further assembled by cellular processing into higher-order configurations. This hierarchical processing is important in the application of molecular tectonics in biomineralization: organic architectures produced by molecular processes are used in turn as the building framework for the synthesis of organized materials, which in turn provide the prefabricated units of complex microstructures.

The three constructional process of biomineralization—supramolecular pre-organization, interfacial molecular recognition (templating) and cellular processing—represent a sequence of increasing tectonic complexity. A commonplace analogy is house building. A building site is established by defining the size and shape of the individual plot (pre-organization). A framework is then laid using a plan (templating) which determines the basic architecture of the dwelling. The house is now built in line with the predefined specifications such as bay windows and gable roofs. If a series of building sites are established in a particular locality, the result is not only the predetermined construction of individual houses but the assembly of houses into a street (processing). Of course, streets can be organized into housing estates, villages, towns, and so on, so the level of organizational complexity can be very large.

Supramolecular preorganization. The initial stage in the fabrication of biominerals is the construction of an organized reaction environment before mineralization. Two general approaches have evolved; (1) the self-assembly of enclosed protein cages and lipid vesicles, and (2) the facilitated construction of extended protein–polysaccharide networks. The former is prevalent in intracellular biomineralization, whereas the latter is predominant in the extracellular (intercellular) spaces generated by multicellular organisms. Both systems produce architectures representative of first-order molecular tectonics.

Vesicles are ubiquitous in the cytoplasm of eukaryotic cells, where they are involved in a wide range of transport, storage and secretory functions. Not only are the vesicles assembled with well-defined compositions but they may undergo a variety of specific transactions involving fusion events, notably during protein trafficking between cisterna in the Golgi stack. The molecular construction of these architectures is based on the balancing of hydrophobic–hydrophilic interactions which exist for amphiphilic molecules in aqueous environments. In the absence of external scaffolds, vesicles generally adopt a spherical morphology. With regard to biomineralization, the enclosed intravesicular space provides a means of controlling the size and location of discrete intracellular mineral products, whereas ion pumps in the vesicle membrane are responsible for controlling the physico-chemical conditions (supersaturation, ionic strength, complexation, pH) pertaining to crystal nucleation and growth²⁸. In general, these lipid-based architectures are often labile, dynamic constructions, compared with polypeptide assemblies in which strong intersubunit interactions tend to give rise to rigid compact structures such as globular or linear proteins. The iron-storage protein ferritin is an exception in that it consists of an 8-nm diameter spherical cage formed by the self-assembly of 24 polypeptide subunits²⁹. In the native protein, the cavity is filled with an iron oxide core consisting of no more than 4,500 iron atoms. The polypeptide subunits of mammalian ferritin are of two different types, both of which are based on a bundle of four long helices, a short helix and a long loop³⁰. Self-assembly of the

FIG. 1 Molecular tectonics in biomineralization. *a*, Intracellular magnetite (Fe_3O_4) crystals in a magnetotactic bacterium. The elongated crystals are synthesized within vesicles that control the size and morphology of the inorganic particles. The crystals are assembled into a linear chain to maintain a magnetic dipole long the cell axis. Scale bar, 100 nm. *b*, Intracellular calcite (CaCO_3) crystals formed within the coccolithophorid, *Syracosphaera*. Each crystal is contained within a vesicle and has a morphology based on a tangentially arranged prismatic block and a thin needle-like radial outgrowth. The shape is determined by the pattern of growth of the developing vesicle. The crystals are organized within a radial array to give an elliptical scale (coccolith). Scale bar, 400 nm. (Photograph courtesy of J. M. Didymus, University of Bath.) *c*, Extracellular lorica formed by the unicellular choanoflagellate, *Stephanoea diplocostata* Ellis. The basket consists of curved silica (SiO_2) rods, each of which is formed in an intracellular vesicle and then transported outside the cell wall. The rods are assembled by tentacles to produce a new lorica for the daughter cell



after cell division³⁹. Scale bar, 1.5 μm . *d*, Fractured section of cuttlefish bone showing the lamellar and columnar structural elements containing aragonite (CaCO_3) crystals. Scale bar, 100 μm .

subunits is facilitated by their high propensity to form stable dimer intermediates (through apolar interactions involving the external surfaces of the loop and one of the long helices). Additional intersubunit interactions involving hydrogen bonding, salt bridges and hydrophobic forces generate higher oligomers and stabilize the quaternary structure of the intact shell. Metal ions and small molecules can enter the internal cavity through molecular channels located at the intersubunit junctions.

Extended polymeric networks are often associated with the construction of consolidated biominerals such as bone, enamel and shell³¹, invertebrate teeth³² and siliceous plant materials³³. The hierarchical structure of bone is well documented³⁴. The primary unit is based on the nucleation of calcium phosphate in nanoscale spaces organized within the supramolecular assembly of collagen fibrils. The precise hole arrangement is governed by the strong covalent crosslinks that exist between the triple-helical molecules when they are staggered by 68 nm along their long axis. Details of the assembly of other biopolymeric networks involved in biomineralization are less precise. Many shells and teeth are constructed within frameworks that may be lamellar, columnar or recticular. In each case, there are two important features of the assembly³¹. First, a relatively inert structural frame is built from insoluble macromolecules (hydrophobic proteins, chitin). Second, acidic proteins (rich in aspartic acid, and often in association with sulphated polysaccharides) are assembled on the hydrophobic scaffold. Mineralization then

takes place at the interface between the acidic proteins and aqueous environment.

Interfacial molecular recognition (templating). The second stage in the fabrication of biological minerals involves the controlled nucleation of inorganic clusters from aqueous solution. In this process, the first-order molecular construction of organic supramolecular systems (such as vesicles, ferritin micelles and polymeric networks) provides a framework for the second-order assembly of the inorganic phase. These pre-organized architectures consist of functionalized surfaces that serve as templates for inorganic nucleation. Although details are not yet available, it is considered that the assembly of mineral nuclei is generally governed by electrostatic, structural and stereochemical complementarity at the inorganic-organic interface³⁵. This aspect of molecular tectonics, in which interfacial molecular recognition assists the construction of nuclei (often with specific crystallographic structures and orientation), is not only a central feature of controlled biomineralization, but has important generic implications in synthetic materials chemistry.

The extent of interfacial complementarity appears to be variable. In enclosed systems such as ferritin, the level of recognition is relatively imprecise because the intrinsic curvature of the organic surface prevails against long-range geometric (epitaxial) interactions. In such systems, redox-active metal-binding sites (ferroxidase centres) and negatively charged patches of glutamate residues (nucleation sites) are involved in generating and

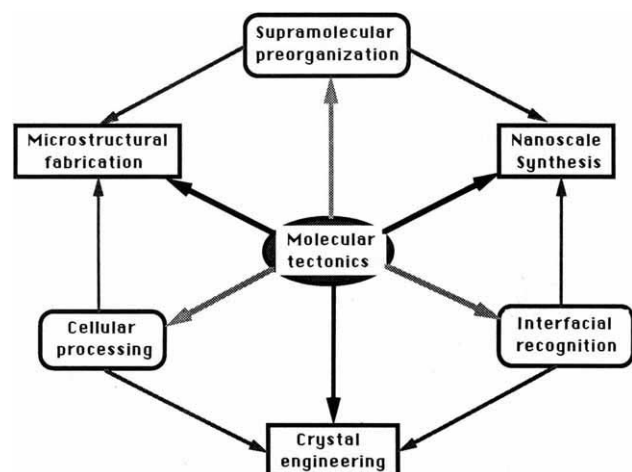


FIG. 2 The connections between molecular tectonics, biomimetalization (rounded boxes) and biomimetic materials chemistry (rectangular boxes). Biomimetic strategies for nanoscale synthesis are based on supramolecular pre-organization and interfacial recognition, crystal engineering is based on the integration of processing and recognition, and microstructural fabrication on the combination of pre-organization and processing.

stabilizing mineral clusters specifically within the protein cavity³⁶. Much of the recognition is based on spatial charge, which directs the accumulation of metal ions, along with associated counter ions, to localized regions of the cavity surface. Similar processes are predicted in lipid vesicles containing charged headgroups and intercalated proteins. In extended

assemblies, by contrast, periodic secondary structures such as antiparallel β -pleated sheets can control the assembly of nuclei along specific crystallographic directions. This is a process of oriented molecular templating. Nucleation of a specific crystal face can occur at the mineral-matrix interface if there is a geometric register between lattice spacings of the crystal structure and distances between ion-binding sites on the organic surface. For example, the spacings between aspartic acid residues deployed along a β -pleated sheet are similar to the Ca-Ca distances in the nucleated (001) face of aragonite crystals comprising the nacreous layer of mollusc shells³⁷.

Cellular processing. The final stage of biomineralization is associated with a variety of constructional processes involving larger-scale cellular activity. In the absence of further cellular intervention, mineral nuclei continue to grow within the confines of their supramolecular hosts. The resulting particles are constrained in size but have normal crystallographic structures and morphologies. In many organisms, however, a third order of tectonic complexity can be established by cellular processing, in which biominerals are endowed with unusual textures and shapes, giving rise to elaborate ultra- and microstructural assemblies. It is at this level of construction that the disparity between biological and synthetic materials is most pronounced.

One of the most striking characteristics associated with many biominerals is their exquisite ornamentation. The subtle and intricate decoration of diatom frustules, coccolith scales and acantharian skeletons arises from the dynamic shaping of vesicles under cellular stresses. Simple elongated morphologies are probably produced by affiliation of the vesicles with cytoskeletal frameworks such as radial (tubulins) or tangential (spectrins) stress filaments. Construction of long vesicles against a cell wall is common, for example, in the production of elongated magnetite crystals in magnetotactic bacteria³⁸ and silica rods in

TABLE 1 Biomimetic approaches in inorganic materials chemistry

Approach	Strategy	Product	Systems	Materials	Refs		
Nanoscale synthesis	Host-guest	Clusters	Reverse micelles	CdS,	50, 51		
			Microemulsions	Pt, Co, metal borides	52		
		Nanoparticles	Vesicles	Fe ₃ O ₄ , CaCO ₃	53, 54		
				Pt, Ag	55, 56		
				CdS, ZnS	57, 58		
				Ag ₂ O, FeOOH, Fe ₃ O ₄ , Al ₂ O ₃	59–61		
				Ca phosphates	62		
				Ferritin	MnOOH, UO ₃ , FeS, Fe ₃ O ₄	63, 64	
				LB films	CdS	65	
				Polystyrene resin	γ-Fe ₂ O ₃	66	
				(γ-EC) _n G peptides	CdS	15	
Crystal engineering	Ligand capping	Single crystals	Monolayers	NaCl, CaCO ₃ , BaSO ₄ , PbS	67–71		
			PolyAsp/polystyrene	CaCO ₃	72		
	Templating	Shaped composites	Tubules	Cu, Ni, Al ₂ O ₃ , Fe oxides	73–75		
			Bacterial fibres	Fe ₂ O ₃ , CaCO ₃ , CuCl	76		
			Bacterial rhabidosomes	Pd	77		
			S-layer proteins	Ta/W	78		
			Directed growth	Textured crystals	Sea urchin proteins	CaCO ₃	79, 80
					Polyanionic peptides	CaCO ₃	81
	Microstructural fabrication	Extended frameworks	Mineral-polymer composites	Polystyrene-butadiene	CaCO ₃ , CdS, Ca phosphate	82	
				Polyvinylchloride	TiO ₂	83	
				Polyacrylate films	Fe oxides, BaTiO ₃	84	
Polysiloxanes				CaCO ₃ , CaSiO ₃	85		
Polyethylene oxide				CdS	86		
Collagen				Ca phosphate	87		
Assembly		Organized materials	Monolayers/Au	CdS	93		
			Monolayers	Fe ₂ O ₃	94		
			Cast bilayers films	Fe ₃ O ₄	95		
			Hydroxyethyl cellulose	CaCO ₃	96		
			Polyacrylate sols	BaSO ₄	97		
SiO ₂ /OH ⁻ gel	CaCO ₃	98					

choanoflagellates³⁹. In the latter case, individual vesicles are stretched out to such an extent that each associated mineral rod has a radius of curvature determined by the shape of the external cell membrane. The architecture of diatom frustules is generated in a similar fashion, except that the vesicles are no longer one-dimensional but extend into a two-dimensional network against the cell wall⁴⁰. More complex shapes, such as the single-crystal calcite elements of coccolith plates, are produced by a combination of radial and tangential morphogenetic processes that are often species-specific⁴¹.

The cellular processing of biomineral shapes often reflects a compromise between the constraints of crystal physics and the genetic manipulation of morphology. For example, crystalline minerals such as calcite (CaCO_3) show preferential growth directions and habits; this anisotropy can be used by organisms in the construction of elongated architectures (for example spicules) in which the direction of fast growth (the *c*-axis) is aligned with the morphological extension (stress filaments) of the vesicle system. In other cases, the genetic patterning of vesicle morphogenesis offsets the intrinsic crystallographic symmetry to produce complex shapes (for example coccoliths) that bear no resemblance to the underlying crystal structure. Other organisms, such as acantharians⁴², fabricate biomineral architectures that integrate structural units based on both crystallographic and biological constraints.

The shaping of individual biominerals is often accompanied by their assembly into organized (intracellular) ultrastructures. Intracellular constructions can be either static or dynamic. An example of an organizational motif that is relatively rigid and spatially fixed is the linear assembly of a chain of membrane-bound magnetite crystals in magnetotactic bacteria (Fig. 1a).

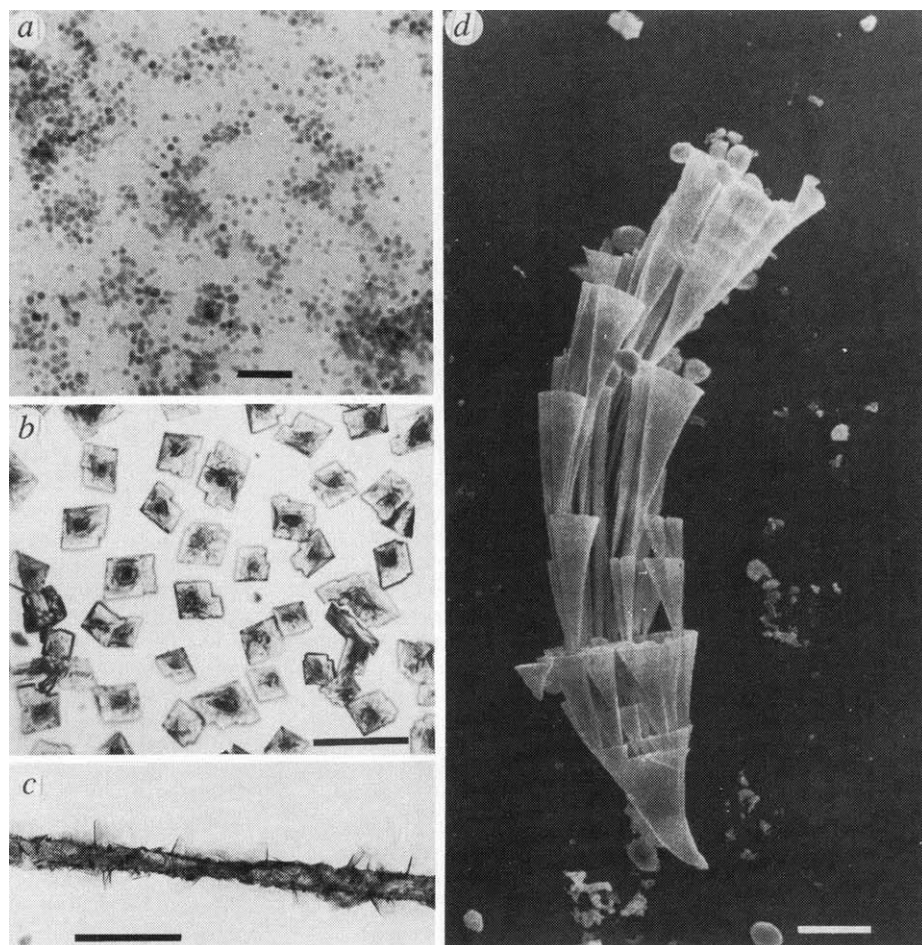
Similarly, calcite crystals are organized in radial arrangements to produce coccolith scales (Fig. 1b). In this latter system, however, the individual scales are exocytosed and assembled into a coccosphere that surrounds the cell wall of the alga. Likewise, individual choanoflagellate cells construct open-ended silica-framed extracellular baskets (lorica) by transporting and assembling over 150 intracellular silica rods³⁹ (Fig. 1c). These feats of microscale structural engineering are staggering, and must represent highly specific processes of molecular recognition and adhesion of preformed surfaces.

Macroscopic organized architectures such as bone, shells and teeth, are associated with extended extracellular matrices. The hierarchical structure of bone results in a composite material capable of being remodelled by active cellular processing in response to mechanical stress. Another feature of these systems is slow repetitive processing. For example, the organized architecture of cuttlefish bone (Fig. 1d) is constructed from the episodic deposition and processing of an extracellular polymeric (chitin) matrix⁴³. Similarly, in the nacreous layer of shells, sheet-like organic assemblies are secreted periodically beyond the mineralization front so that progressive infilling results in the construction of a highly organized lamellar architecture⁴⁴.

Biomimetic materials chemistry

Can a molecular-tectonics perspective of biomineralization be employed in materials chemistry? Clearly, the generic concepts of molecular recognition, pre-organization, templating and processing are common to both fields. But can one go further and demonstrate that materials synthesis can be based on a biomimetic approach? It seems sensible to adopt a rather loose definition for the term, biomimetic materials chemistry, because there

FIG. 3 Examples of biomimetic inorganic materials. *a*, Ferrofluid containing nanoscale magnetite crystals, formed within the cavity of individual ferritin molecules⁶⁴. The mean core diameter is 4 nm. Scale bar, 40 nm. (Photograph courtesy of T. Douglas, University of Bath.) *b*, Array of oriented calcite crystals nucleated under a stearic acid monolayer formed at the air-water interface⁶⁹. Scale bar, 100 μm . *c*, Mineralized cylinders of self-assembled galactocerebroside tubules formed by template-induced nucleation of iron oxides⁷⁵. Scale bar, 400 nm. *d*, Organized aggregate of BaSO_4 crystals formed from aqueous supersaturated solution in the presence of polyacrylic acid (J. P. Hopwood and S. M., unpublished observations). The microstructure consists of a series of cone-shaped elements that are preferentially aligned. The architecture develops by the episodic nucleation of new cones around the rims of mature elements. Each cone is constructed from a close packed array of fibre-like crystals emanating from a consolidated basal structure. Scale bar, 10 μm . (Photograph courtesy of J. D. Hopwood, University of Bath.)



is much conceptual overlap between those approaches inspired by biology and those based on molecular engineering in materials chemistry. One major concern is how to translate the harsher regimes of current fabrication technology into the mild (low temperatures and pressure) chemical conditions characteristic of biomineralization. Furthermore, biomineralization is associated with an absence of toxic intermediates, and we could do well to incorporate this biological rule of construction in assessing the impact of future materials fabrication processes on the environment.

Biomimetic approaches in materials chemistry are at present primarily focused on the study of processes and properties at the inorganic–organic interface^{45–48}. These strategies can be embraced within three activities—nanoscale synthesis, crystal engineering and microstructural fabrication—that are related to different combinations of the three biological constructional processes described above (Fig. 2).

Nanoscale synthesis. Because nanoscale particles show quantum size effects in their electronic, optical and chemical properties, there has been much activity in this area of synthetic materials chemistry⁴⁹. A complementary biomimetic strategy involves the use of organic supramolecular cages that act as spatial hosts and reactive interfaces for inorganic guest materials. Reverse micelles^{50,51}, microemulsions^{52–54} and surfactant vesicles^{55–62} have been used in a number of studies involving semiconductor, catalytic and magnetic materials (Table 1). These systems often have a limited stability with regard to aggregation and hydrolysis, although the use of polymerized vesicles has ameliorated some of these problems. An alternative approach is to use the biomolecular cage of the iron-storage protein ferritin as a more robust environment for inorganic materials synthesis^{63,64}. *In situ* chemical reaction of the native iron oxide cores readily produces nanoscale iron sulphides, whereas the reconstitution of empty protein cages in aqueous salt solutions gives a range of non-native oxide materials, one of which, magnetite (Fe₃O₄) (Fig. 3a) endows the protein with permanent magnetic properties⁶⁴. Other approaches that are not strictly biomimetic in design (for example, the intercalation of nanoscale particles of CdS in Langmuir–Blodgett films⁶⁵ and magnetic iron oxides in polystyrene resins⁶⁶) serve to illustrate the general importance of inorganic–organic interactions in the control of materials synthesis.

Crystal engineering. Controlling the nucleation and growth of inorganic materials is an important consideration in materials and colloidal chemistry. Oriented inorganic substrates such as Au and Si are used as epitaxial surfaces in chemical vapour deposition methods, and it would be significant if similar processes could be routinely undertaken in aqueous solutions. Over the past few years, a biomimetic approach to crystal engineering has been developed (Table 1). For example, compressed Langmuir monolayers have been used as nucleation substrates for the oriented growth of inorganic materials^{67–71}. One particular advantage of this approach is that the functionality and packing of the organic surface can be readily modified. Indeed, in many instances, complementarity between the surface chemistry and structure of the surfactant film, and crystal faces of the incipient nuclei, results in the oriented nucleation of two-dimensional arrays of discrete crystals at the monolayer solution interface (Fig. 3b). Another approach, involving the adsorption of polyaspartate onto a sulphonated polystyrene surface⁷², was also effective in controlling oriented nucleation. These experiments, like those involving monolayers, highlight the requirement for molecular periodicity because the adsorbed molecules must adopt a β -sheet conformation if oriented nucleation is to be induced.

Biomolecular surfaces, such as tubules^{73–75}, bacterial fibres⁷⁶, rhabdosomes⁷⁷ and S-layer proteins⁷⁸, have been used as structural templates to guide the deposition of inorganic materials within predetermined spatial patterns (Table 1). The resulting crystals are often randomly arranged, but the microscopic mor-

phology is controlled. In particular, elongated fibrous inorganic–organic composites can be formed (Fig. 3c). The crystal morphology and texture of inorganic materials can also be influenced by soluble biomolecules. For example, acidic macromolecules (isolated from sea urchins) influence the morphology and fracture of synthetic calcite crystals by surface recognition and site-specific occlusion, respectively^{79,80}. Studies using synthetic-peptide analogues of these biomolecules suggest that polyanionic domains of polyaspartate and serine phosphate are responsible for the functional activity of the shell proteins⁸¹.

Microstructural fabrication. Controlling the microstructure of biominerals by cellular processing is clearly the most difficult aspect of molecular tectonics to translate into the world of materials chemistry. The sophisticated use of cellular activity in shaping and assembling biominerals is difficult to reconcile with the static methods of chemical fabrication. Some progress has been made, however, in the use of functionalized polymers^{82–85}, polymer gels⁸⁶ and biomolecular matrices⁸⁷ as organized frameworks for *in situ* inorganic precipitation (Table 1). One of the main problems is the low mineral content of the resulting inorganic–organic composites⁸⁸. One possible way round this is to run the system in reverse, that is, to incorporate a soluble polymer (polyethylene oxide) into a preformed mineral matrix (such as the interlayers of a mica⁸⁹) or alternatively, precipitate both mineral (calcium silicate/aluminate) and polymer (polyvinyl alcohol) simultaneously^{90,91}. It will be interesting to determine how the mechanical properties of these organo-ceramics compare with those observed in biominerals such as abalone shell⁹².

Finally, how can inorganic materials be assembled by molecular tectonics into organized long-range architectures? There can be no doubt that this remains a major challenge. Recently, self-assembled 1,6-hexanedithiol monolayers on gold surfaces have been used to organize the chemisorption of preformed CdS particles from solution⁹³. A similar process has been observed with conventional monolayers in the presence of aqueous-dispersed iron oxide crystals⁹⁴. Alternatively, lamellar arrangements of inorganic particles can be induced by casting films of metal-ion-containing lipid vesicles onto solid substrates⁹⁵. Although the level of materials organization is not high, these studies suggest that organized materials can be fabricated if we can develop appropriate recognition processes between the preformed constituent units. We should also not rule out the possibility of using dissipative systems, in which spatial and temporal organization is established by chemical diffusion and associated feedback loops. Crystallization in viscous media may be one such system. For example, we have recently observed elaborately organized architectures of BaSO₄ crystals grown from supersaturated solutions containing specific concentrations of polyacrylic acid (J. D. Hopwood and S.M., unpublished observations) (Fig. 3d). Similar observations have been made with other polymers and crystallization systems^{96,97}, as well as in alkaline silica gels⁹⁸ (Table 1), although the molecular details of these constructional processes are unknown. Perhaps we need to look to the precise biological mechanisms of vesicle targeting and fusion for inspiration? These processes involve vesicle fusion proteins (NSF and SNAP) and associated (SNARE) receptors⁹⁹. Could we construct inorganic–organic architectures from the controlled aggregation of mineralized supramolecular assemblies primed with appropriate attachment and receptor sites? The construction of a simple linear chain of mineralized units, analogous to the organizational motif observed in magnetotactic bacteria, would be a bold beginning.

Discussion

In this article I have attempted to indicate how the science of chemical construction of organized architectures—molecular tectonics—is a unifying principle which links biomineralization and material chemistry. Moreover, understanding how biology constructs organized inorganic materials by molecular processes

will inevitably result in innovations in synthetic systems, even if these are only loosely affiliated with direct biomimicking. Biomimetic materials chemistry should be viewed as a malleable science capable of being shaped by inspirations emanating from both physical and biological sources. It is an eclectic discipline, combining a knowledge and practice of fields such as organic supramolecular chemistry, molecular recognition, nanotechnology, biomineralization, crystal and colloidal chemistry, polymer chemistry and materials processing.

At present, significant developments have been made in biomimetic approaches based on the combination of interfacial recognition with either supramolecular pre-organization (nanoscale

synthesis) or external processing (crystal engineering). Few advances have been achieved in the controlled microstructural fabrication of mineral-polymer composites, although several innovative strategies are being undertaken. A major triumph will be the incorporation of molecular biology into biomimetic inorganic materials chemistry because functional templates and reaction cages could, in principle, be routinely produced. Unfortunately, we have at present insufficient knowledge about most of the biological systems to initiate such an approach. □

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Seismic constraints on mantle flow and topography of the 660-km discontinuity: evidence for whole-mantle convection

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Recent seismic models of three-dimensional mantle structure and topography on the transition-zone seismic discontinuities permit the direct evaluation of the buoyancy forces that drive large-scale mantle flow. This buoyancy distribution, coupled with radial viscosity models that are consistent with the observed geoid, predicts flow patterns with significant vertical flow through the 660-km phase boundary. The observed topography on the 660-km discontinuity acts to inhibit convection across the boundary, but is two to four times too small to prevent whole-mantle convection.

A FUNDAMENTAL issue in mantle convection is whether large-scale mantle flow is stratified by changes in mineral phase or bulk chemistry across the seismic discontinuity at 660 km¹⁻⁴. This question has major implications for the potential mass and heat transfer throughout the mantle which strongly affect its chemical and thermal evolution⁵. Seismic imaging of subducting slabs has not resolved this issue, with some studies indicating slab penetration below the 660-km discontinuity^{6,7}, whereas others have found horizontal seismic velocity anomalies in some areas that suggest the slabs are deflected by the interface^{8,9}. This debate has recently been reinvigorated by three-dimensional numerical experiments of mantle convection that include the effects of a phase change at 660 km, experiments that predict the possibility of significant amounts of flow stratification due to the negative Clapeyron slope of this phase transition¹⁰⁻¹³, and an analysis that finds no change in the pattern of three-dimensional seismic velocity structure near 660-km depths as would be expected for layered convection¹⁴.

Here we present a new approach to this problem. We use seismic tomographic maps of three-dimensional mantle velocity structure¹⁵⁻¹⁸ and maps of the topography on the 410- and 660-km discontinuities^{19,20} to infer directly the density distribution that drives large-scale mantle flow (the flow is caused by the buoyancy forces that arise from lateral density gradients). We explore the effects of this density distribution on mantle flow for several plausible radial viscosity structures^{21,22} that have been proposed to fit the observed geoid, given the seismically inferred densities. The resulting vertical flow pattern for each viscosity model shows directly the amount of vertical mass transfer across the 660-km boundary as well as flow rates at other mantle depths. If mantle flow is layered as sketched in Fig. 1, the mean radial flux should show a strong reduction near 660-km depths¹¹. In fact we see no apparent cusp at any depth in the curves of radial flux against depth, implying a coherent low-order pattern of whole-mantle flow. The observed topography on the 660-km discontinuity acts to oppose convection but is too small to prevent vertical flow. These results do not depend on the assumed Clapeyron slope of the 660-km phase change, and are robust with respect to reasonable variations in mantle viscosities and the viscosity-to-density scaling relationship.

Seismic models of 3-D mantle structure

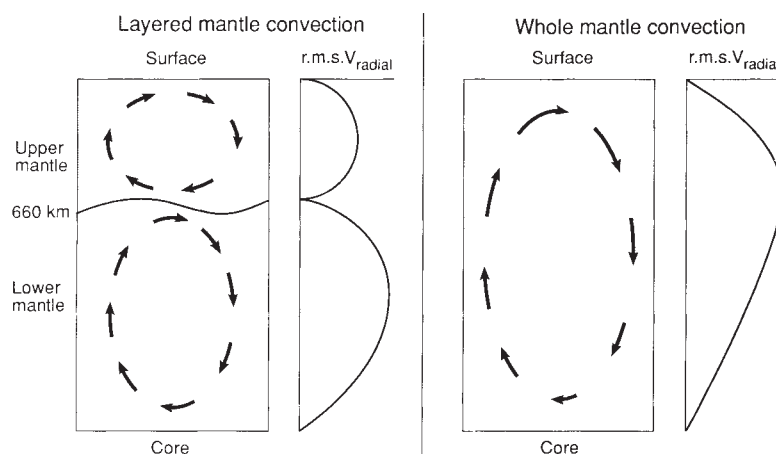
Recent tomographic models of mantle shear-wave velocities now provide good global-scale information on the mantle density structure. Here we use two of these recent models, SH8WM13 (refs 17, 18) and SH10c (ref. 15) which, though based on mostly

different data, show generally good agreement between their velocity structures. These seismic inversions recover a three-dimensional image of aspherical variations in seismic shear velocity β . Converting these velocity perturbations to aspherical density variations requires some assumptions. In regions where thermal effects are dominant (that is, compositional effects are small, see below), $\delta\beta$ is typically less than $\pm 2\%$ of the mean shear-wave velocity, and can be related to aspherical density variations by the linear approximation at constant pressure $\delta\rho = (\partial\rho/\partial\beta)_P\delta\beta$. The primary uncertainty is to what extent an increase in pressure reduces $(\partial\rho/\partial\beta)_P$. Laboratory measurements and seismic observations of slab anomalies⁷ suggest that $(\partial\ln\rho/\partial\ln\beta)_P \approx 0.4$, with little depth dependence in the upper mantle. Although recent laboratory measurements predict $(\partial\ln\rho/\partial\ln\beta)_P$ to be relatively pressure-insensitive, Debye-Grüneisen theory suggests that it may decrease substantially with increasing pressure²³, and several models^{21,24} have incorporated this assumption in determining radial viscosity models that fit the observed geoid with a seismically inferred density structure, using values of $(\partial\ln\rho/\partial\ln\beta)_P$ that vary from ~ 0.2 – 0.4 at upper mantle/transition-zone depths to ~ 0.1 in the lower mantle. Here we will explore the mantle flow predicted by two different viscosity models, one of which assumes that $(\partial\ln\rho/\partial\ln\beta)_P \approx 0.4$ throughout the mantle, whereas the other assumes that $(\partial\ln\rho/\partial\ln\beta)_P$ decreases from 0.2 in the upper mantle to 0.1 in the lower mantle (see Fig. 2). For consistency, we always use the velocity–density scaling appropriate for each model.

Topography on the 660-km discontinuity

The remaining mass anomalies that can drive mantle flow are due to vertical deflections of internal density discontinuities associated with chemical or phase traditions. Mineral physics experiments indicate that the 410-km discontinuity is caused mainly by the phase change between α - and β -olivine, and the 660-km discontinuity is mainly due to the transformation γ -spinel $\rightarrow$ perovskite + magnesiowüstite. The 410-km phase change is found to be an exothermic reaction with a positive Clapeyron slope (dP/dT) which should cause the discontinuity to move up in response to reduced temperatures. In contrast, the 660-km phase change is an endothermic reaction with a negative Clapeyron slope and should move down in colder regions. The 660-km discontinuity will tend to resist the penetration of both cold descending material or hot ascending material because the phase boundary is perturbed in the direction of flow and the restoring force induced by the density perturbation will oppose this flow. The situation is reversed for the 410-km discontinuity in which

FIG. 1 Pattern of radial mantle flow implied in layered- and whole-mantle convection patterns. The radial flux across the 660-km discontinuity is a strong discriminant for the pattern of mantle convection.



the phase change acts to encourage thermally induced vertical flow.

The 660-km discontinuity is of most interest for this study because it acts to oppose vertical flow and is the depth for the proposed layering between the upper and lower mantle. Seismic observations based on reflected and converted phases suggest that topography on the 660-km discontinuity is less than 30–40 km^{25,26}; this topography has been mapped recently on the global scale required for our analyses using long-period reflections^{19,20}. (If a Clapeyron slope based on laboratory measurements is assumed, then the amplitude of phase-boundary relief could potentially be inferred via the circuit lateral

$\delta\beta \rightarrow \text{lateral } \delta T \rightarrow \text{vertical phase boundary perturbation}$. Here we avoid the uncertainties of this procedure by measuring 660-km topography directly from seismic observations of reflected phases.) Because of the density contrast at 660 km, this topography implies the existence of significant lateral density anomalies at the interface. These density perturbations do not follow the same velocity-to-density scaling as do the thermal buoyancy forces discussed above.

Relief on internal discontinuities will affect a tomographic inversion for aspherical velocity structure if these interfaces are assumed to be flat. We can, however, correct for this effect on our inferred densities by considering the velocity contrasts across the discontinuities. If an interface is perturbed by h in depth, a shear velocity anomaly occurs at the interface of height h and amplitude $\Delta\beta/\beta$, where $\Delta\beta$ is the discontinuity velocity contrast. Assuming $(\partial \ln \rho / \partial \ln \beta)_\rho = 0.4$ (appropriate for the upper mantle), the calculated density anomaly $(\Delta\rho/\rho)_{\text{tomo}} = 0.4\Delta\beta/\beta$. This typically severely underestimates the true $\Delta\rho$ at the interface. For example, at the 660-km discontinuity in PREM (ref. 27), $\Delta\beta/\beta = 6\%$, implying that $(\Delta\rho/\rho)^{660}_{\text{tomo}} = 2.4\%$, in contrast to the PREM $(\Delta\rho/\rho)^{660} = 9\%$. This is why it is necessary to include explicitly the density anomalies (due to boundary perturbations) in the buoyancy calculations, after first correcting for the small amount of the density anomaly which is expressed in the tomography results. Realistically, the limited vertical resolution in the tomographic inversion will cause the observed velocity anomalies due to discontinuity topography to be smeared out in shape and reduced in amplitude. The above correction is, however, still valid as our results do not depend very much on the exact depth and magnitude of the density anomalies, but only on their vertically integrated values which are better determined in tomographic inversions.

Geodynamic models of mantle flow

As discussed above, seismic methods can determine directly the large-scale aspherical mantle density structure that drives mantle flow. This density map, together with the observed geoid, can be inverted to give the radial viscosity structure of the mantle, one of the major geodynamic modelling achievements of the 1980s^{21,22,24,28–33}. Recent geoid/tomography inversions crudely agree on the radial mantle viscosity structure^{21,22,24,31}. In general, the upper mantle is about 10–30 times less viscous than the lower mantle, which has a viscosity of $\sim 10^{22}$ Pa s. Two recent models of radial mantle viscosity structure by Hager and Richards²¹ (H–R model) and King and Masters²² (K–M model) are shown in Fig. 2. The two models represent a range of plausible radial mantle viscosity structures. (Note that geoid fitting does not constrain the absolute magnitude of the mantle viscosity, only radial variation up to a multiplicative constant.) These models require different assumptions about velocity–density scaling

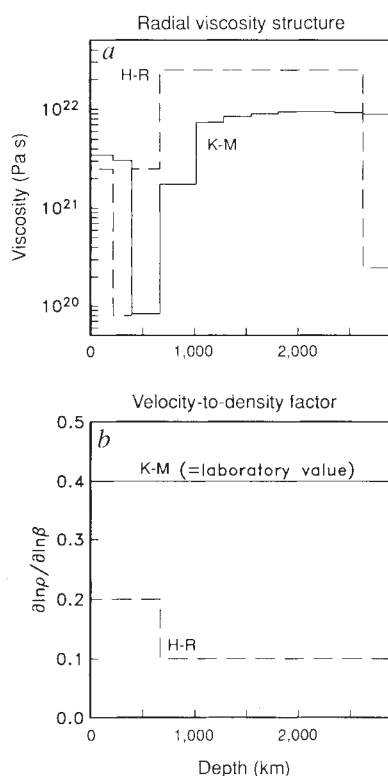


FIG. 2 a, Radial viscosity structure for two mantle viscosity models, H–R (Hager and Richards²¹) and K–M (King and Masters²²), which successfully predict the observed geoid from buoyancy forces imaged by aspherical seismic velocity inversions. b, Corresponding velocity-to-density scaling required by each model to fit the observed geoid.

(Fig. 2b) to fit the peak-to-peak magnitude of the geoid; in particular, the H-R model requires that $(\partial \ln \rho / \partial \ln \beta)_p \approx 0.1$ in the lower mantle (similar models are determined by Forte *et al.*²⁴), whereas the K-M model assumes that $(\partial \ln \rho / \partial \ln \beta)_p \approx 0.4$ throughout the whole mantle (similar models are determined by Ricard and Wumling³¹). For each viscosity model (and its corresponding velocity-density scaling) and the buoyancy distributions implied by each seismic model (and the seismic model's 'corrected' 660-km relief²⁰), we solve for large-scale mantle flow assuming incompressible, self-gravitating viscous mantle flow²¹.

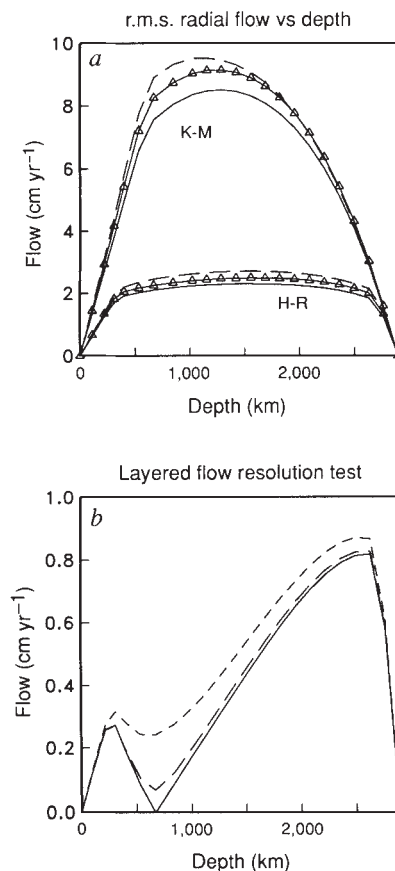
Figure 3 shows the mean radial flow distribution that is predicted for the H-R and K-M models with the buoyancy forces implied by seismic models SH8WM13 and SH10c. Neither model shows a significant decrease in radial velocity near the 660-km discontinuity that would favour layered convection. The inclusion of the density anomalies due to the observed 660-km topography generally reduces velocities throughout the mantle (as would be expected if this barrier tends to oppose, but not stop downwelling at subduction zones), but still does not cause a local minimum in the r.m.s. velocity near the 660-km interface. Figure 4 shows the radial velocity distribution calculated from the H-R model, the SH8WM13 aspherical seismic velocity model, and Shearer's determination of 660-km relief²⁰ shown in Fig. 5a. The resulting mantle flow has an upwelling and downwelling pattern that differs little from mid-mantle to lower-mantle depths—that is, this flow structure is strongly coherent from one depth to another.

Some controversy exists regarding the radial resolution of the seismic velocity models, particularly in the vicinity of the 660-km discontinuity. Fortunately our results are not very sensitive to errors in the radial location of seismic velocity anomalies. For the low-order structure that dominates the seismic models, the flow near 660 km is driven by the effect of velocity perturbations

averaged over 2,000 km or more in depth. There is a strong smoothing effect on the mantle flow from the buoyancy distribution that drives it: flow at a given depth responds in nearly the same manner to a density anomaly that is close in depth as it does to a similar density anomaly that is much deeper or shallower. (For example, the H-R response for flow at 660-km due to a degree-four mass anomaly at 2,000 km is only 35% less than that due to a mass anomaly at the 660-km interface itself. The response to lower-degree mass-anomalies is even less depth-sensitive.) Our predicted mantle flow pattern is determined largely by the vertically integrated effect of velocity anomalies in the mid-mantle. These large-scale radial averages of the tomography models are much better determined than the values at particular depths, because the seismic travel times used to derive the velocity models produce a similar smoothing effect.

To be sure that our method is capable of detecting a kink in the r.m.s. flow field if it existed, we created some hypothetical density distributions which predict such a kink even in the absence of any discontinuity topography. Our starting point is the calculated 660-km discontinuity topography (see below) required to stop flow across the discontinuity for the SH8WM13 model and H-R viscosity model. This introduces 9% density anomalies at 660 km, which are sufficient to counter the other buoyancy forces in the mantle to enforce layered convection. Thus, a model with intrinsic density anomalies (as opposed to boundary deflections) of this size at 660 km would also result in layered convection, and require no discontinuity topography. The predicted r.m.s. flow for this model is shown as the solid line in Fig. 3b, and contains a null point at 660 km. However, the low radial resolution of the seismic tomography will smear out a pronounced density anomaly right at 660 km, so we experimented to see how this would affect our results. The dashed line in Fig. 3b shows the r.m.s. flow predicted when the mass anomalies are uniformly smeared over 600 km; the dotted line shows

FIG. 3 a, R.m.s. radial mantle flow pattern with depth that is predicted by several density and viscosity hypotheses. The top three profiles are for the K-M model, and the bottom three are for the H-R model. For each model, the solid line is for the SH8WM13 density model, a PREM density jump at 660-km, and the map of 660-km relief²⁰ shown in Fig. 5. Triangles show radial flow patterns for buoyancy distributions that do not include the effects of the 660-km relief; these models have generally higher flow rates throughout the entire mantle. Dashed lines show models that include the effects of 660-km topography and the SH10c buoyancy distribution. All models show no hint of a significant reduction in vertical flow through the 660-km interface that would indicate layered mantle convection as sketched in Fig. 1. b, Results from a synthetic resolution test where, for the SH8WM13 density model and H-R viscosity model, the mass anomaly at 660 km needed for layered convection (shown in Fig. 5) is added to the SH8WM13 density field. The solid line shows the predicted r.m.s. radial flow when this additional mass anomaly is concentrated at 660 km, the dashed line shows the r.m.s. flow diagnostic when the mass anomaly is uniformly smeared over 600 km (from 360 to 960 km), and the dotted line shows the r.m.s. diagnostic when the mass anomaly is smeared over 1,200 km (from 100 to 1,300 km).



the result when the anomalies are uniformly smeared over 1,200 km. In each case there is a pronounced kink in the r.m.s. flow near 600 km. Most discussions of the vertical resolution of the tomography models suggest resolving lengths of 400 km or better, but even if the resolutions were as poor as 600–1,200 km, our method would still produce a kink in the r.m.s. flow for these models. In fact, these density distributions, when converted to S-velocity anomalies, predict travel-time residuals for vertically travelling S-waves of 5 s (K–M model) to 7 s (H–R model). These are big anomalies, which would be detected easily by seismic means.

The mantle flow rates predicted by the H–R model are large, $\sim 4 \text{ cm yr}^{-1}$, and the K–M model predicts even larger vertical flow rates. Although lower-mantle horizontal flow rates are typically half as large as vertical flow rates, these speeds still seem relatively high: mean vertical flow rates of 4 cm yr^{-1} imply a cycle time of $\sim 150 \text{ Myr}$ to traverse the mantle. Although the geoid is insensitive to the absolute magnitude of the mantle viscosity, flow rates scale to the reciprocal of viscosity. Increasing all viscosities in the H–R model by a factor of two would still generate an upper-mantle viscosity structure that is consistent with glacial rebound constraints while cutting mean vertical flow speeds in half. Increasing all viscosities in the K–M model by a factor of six to ten would lead to comparable lower-mantle vertical flow rates of $\sim 1\text{--}2 \text{ cm yr}^{-1}$. Note that scaling a mantle viscosity model by a constant factor does not change the shape, but only the absolute magnitude of the predicted flow field; our conclusions regarding whole-mantle versus layered-mantle convection are not affected.

Figure 5a shows the observed topography on the 660-km discontinuity²⁰ after correcting for the effects of lateral velocity

variations in the mantle. Regional depressions are seen in the western Pacific, South America and the North Atlantic, with highs near Africa and Eurasia; peak-to-peak depth variations are about 32 km. Figure 5b and c shows the topography on the 660-km discontinuity that is needed for layered mantle convection assuming the SH8WM13 mantle heterogeneity model (similar relief structures are seen for the SH10c model). Although these patterns are similar in shape to the observed topography, the H–R model (Fig. 5b) requires peak-to-peak depth variations in the interface of about 65 km, whereas the K–M model (Fig. 5c) needs depth variations of 130 km. Thus, our results show that the observed topographic variations in the 660-km discontinuity are from two to four times too small to prevent whole-mantle convection.

Implications for mantle dynamics

The general correlation between the observed 660-km interface deflection and the flow pattern seen in Fig. 4 is to be expected. Subduction zones should represent regions where cold material is advected downwards, thereby depressing an endothermic phase boundary. In contrast, upwelling regions are likely to have elevated temperatures that raise the depth at which the phase transition takes place. This should also cause a correlation between the maps predicted to enforce layered flow and seismic observations of 660-km relief. Figure 5 shows that there is only rough spatial coherence between these patterns. Some of the differences between the patterns are probably due to inaccuracies in the topography model caused by gaps in spatial coverage²⁰, but in several regions the disagreement seems to be real. In particular, North Atlantic and Eurasia are prominent areas where these two pictures are anticorrelated. Note that these are regions where

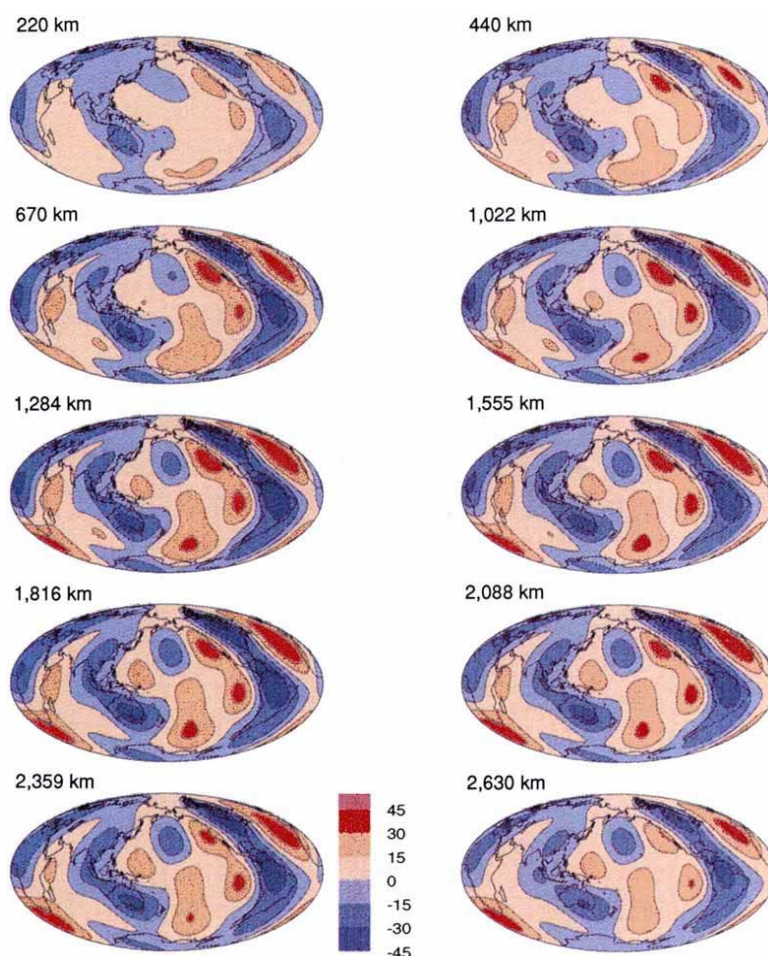


FIG. 4 Radial mantle velocity pattern predicted by the H–R viscosity structure, buoyancy forces due to 660-km topography with a PREM density jump, and the SH8WM13 buoyancy distribution. Vertical flow rates are shown at ten layers from upper-mantle to lower-mantle depths. Note the strong coherence between upper-mantle and lower-mantle centres of upwelling and downwelling; this flow pattern implies a top-to-bottom of large-scale mantle circulation. Velocity scale is in mm yr^{-1} .

active mantle flow may be relatively slow, so that the lateral temperature structure (which integrates a long previous flow history) is not in phase with the vertical flow pattern (which is a response to the current buoyancy distribution).

An advantage of our approach is that our results do not depend upon the assumed Clapeyron slope, γ_{660} , of the 660-km phase transition. Nevertheless, we can calculate an approximate value of γ_{660} from the seismic tomography and discontinuity topography observations. Velocity perturbations at 660 km in the three-dimensional mantle models are correlated approximately with the pattern of the topography seen in Fig. 5. The SH8WM13 model has peak-to-peak variations in shear velocity of 2.4% at 660 km, whereas the SH10c model has 3.7% variations. If we assume a scaling between shear velocity and temperature, $\partial\beta/\partial T$, that is consistent with laboratory measurements and seismic observations of slab anomalies^{7,26}, then an increase in shear velocity of 1% corresponds to a change in temperature of 100 °C. To fit the observed peak-to-peak topography of 32 km, γ_{660} ranges from -3.4 to -5.3 MPa per °C for the SH10c and SH8WM13 models. These numbers are in reasonable agreement with recent laboratory measurements³⁴ of the γ -spinel→perovskite+magnesiowüstite phase change which indicate $\gamma_{660} = -4 \pm 2$ MPa per °C. This is encouraging as the peak-to-peak amplitudes in the seismic models can be influenced by the degree of horizontal smoothing imposed during the inversion—the consistency between predicted and measured values of γ_{660} suggests that this is not a problem in our analysis.

Given that our results clearly indicate whole-mantle flow at $\gamma_{660} \approx -4$ MPa per °C, it is puzzling that recent numerical simulations of convection^{10–12} suggest that layering or intermittent mixing can occur at values of γ_{660} between -2 and -4 MPa per °C (convection is inhibited more at steeper values of the Clapeyron

slope because the discontinuity is perturbed more for a given temperature anomaly). Possible explanations for this discrepancy include temperature-dependent viscosity in the mantle and the large lateral scale of the velocity anomalies in the tomography studies. The numerical studies assume isoviscous, or simply layered, viscosity structures, whereas the actual mantle flow involves a temperature-dependent mantle rheology which can maintain much larger lateral temperature structure. Large lateral density anomalies will pass through the 660-km discontinuity more readily than small anomalies of the same amplitude, because it is more difficult for the material to be dispersed horizontally. Such a wavelength dependence in the vertical flow has been noted in numerical modelling experiments¹². The very large horizontal scale of the anomalies seen in the tomographic images are a critical factor in our buoyancy calculations which implies whole-mantle convection. If the features were of much smaller wavelength then it is likely that layered convection could result.

Thus, it is important to consider the extent to which the lack of resolution in the seismic models is affecting our results. The models express velocity perturbations in spherical harmonics up to degrees eight to ten, limiting the horizontal resolution of heterogeneity to wavelengths of ~4,000 km or longer. But the spectrum of heterogeneity in the models is not flat; most of the power is contained in the first six harmonic degrees, and some seismic results indicate that heterogeneity may remain relatively weak out to degree 36 (~1,000 km wavelength)³⁵. At even shorter scales, neither the global tomography nor the large-scale mantle flow models discussed here realistically images narrow rising plumes or subducting slabs; they are models only of the large-scale background mantle flow. Plumes will have a minor effect on the size of the background flow, the concentrated buoyancy force of a ~100 km diameter plume is relatively small compared

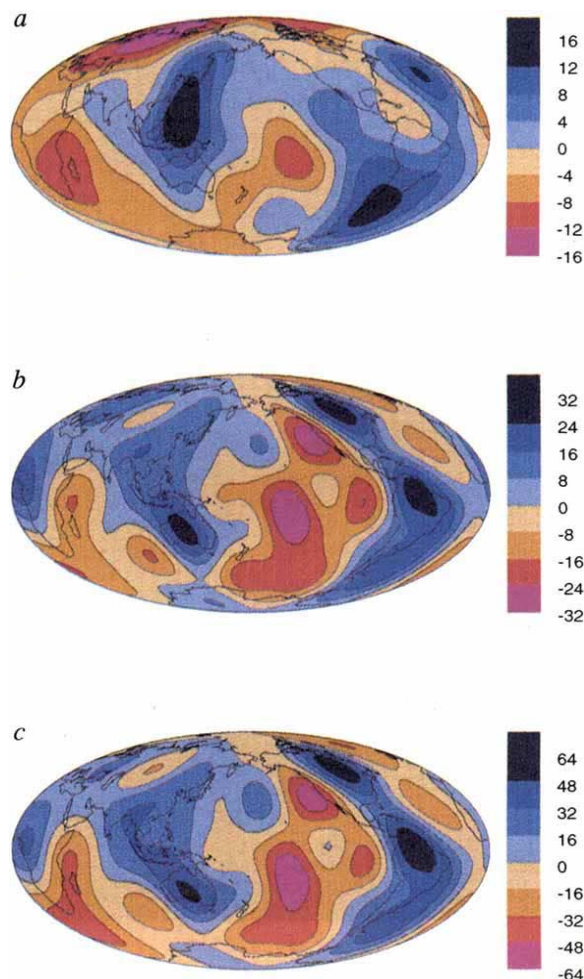


FIG. 5 a, Observed topography at the 660-km interface, calculated using the SH8WM13 model of aspherical seismic velocity structure (the topography calculated using the SH10c velocity model is quite similar). Positive relief corresponds to a deeper phase-boundary interface. b, 660-km relief needed for layered convection (no flow across the 660-km interface) for the H-R model and a PREM jump in density across the 660-km discontinuity (9% increase). This model predicts twice the peak-to-peak topography than is observed. c, 660-km relief needed for layered convection for the K-M model and a PREM jump in density across the 660-km discontinuity. This model predicts four times the peak-to-peak topography than is observed. The major reason for the increase relative to the H-R model is that the K-M model assumes a laboratory scaling of velocity to density in the lower mantle which is four times larger than the H-R value. Depth perturbations are contoured in km, with blue regions corresponding to depressions in the discontinuity.

with the regional buoyancy forces which are included in our calculations, and the regional heating from persistent plume concentrations is probably also incorporated. (For example, a 'hot region' in the South Central Pacific is a persistent feature at all depths in our aspherical density models. This region lies below a major cluster of surface hotspots.) Although the global tomography results do not resolve narrow subducting slabs, they do resolve a cold regional halo about major circum-Pacific subduction zones. If we are underestimating the negative buoyancy in the slabs, this missing buoyancy would act to enhance background downwelling near active subduction zones, implying that these results are a relatively conservative estimate of the forces driving background flow through the 660-km discontinuity.

Descending slabs represent an interesting combination of the factors of size and temperature-dependent viscosity. Their relatively small volume inhibits penetration through the 660-km discontinuity while their colder temperatures make them stronger and more likely to penetrate this discontinuity. This is because the slab will act as a stress guide for the negative buoyancy force above and below the phase transition. The trade-off between these effects may explain why seismic studies⁶⁻⁹ indicate that in some areas slabs penetrate through the discontinuity, whereas in other regions the slabs are deflected by the discontinuity to form large pools of cold material in the transition zone behind the subduction zones (the results discussed here indicate that these regions also eventually sink through the discontinuity).

Validity of modelling assumptions

It is unlikely that a simple velocity-density scaling relationship based on temperature variations is valid throughout the mantle^{36,37}. Instead, there probably exist compositional boundary layers near the surface and within the D'' region at the core-mantle boundary in which density cannot easily be predicted from velocity. To test this possibility, we repeated our flow calculation for the case in which buoyancy forces were removed from both the top 400 km and the bottom 300 km of the mantle, and found nearly the same flow across the 660-km discontinuity. Again, this emphasizes that it is the integrated effect of the

velocity anomalies in the mid-mantle which are most important in determining the large-scale flow pattern.

Another potential weak spot in our mantle flow calculations is the assumed mantle viscosity models. Most of these models were derived to fit the observed geoid by assuming whole-mantle flow. Could alternative viscosity models exist that are consistent with layered-mantle flow? Attempts to produce such models have so far been unsuccessful in fitting the geoid²⁴, or have required a compositional boundary near 660 km with hundreds of kilometres of dynamic topography²¹. As we have discussed, such topography is precluded by seismic observations of discontinuity depths. Thus it seems improbable that a viscosity structure exists which could produce layered flow and remain compatible with gravity and seismic observations. An important next step in mantle viscosity calculations (based on fitting the geoid) will be to incorporate the observed 660-km topography as an additional constraint.

For the results discussed above we have assumed the PREM density contrast of about 9% at the 660-km discontinuity. A larger density contrast would cause the 660-km discontinuity to resist vertical flow more strongly for the same depth perturbation. Thus, whole-mantle convection could conceivably be prevented if $\Delta\rho_{660}$ were large enough. The required values of $\Delta\rho_{660}$ vary from 18% (H-R model) to 36% (K-M model). The PREM density contrast is, however, slightly larger than most other seismic estimates^{26,38,39}; a value of 18% would be difficult, if not impossible, to reconcile with seismic data and mineral physics results for the 660-km phase change.

Our results show that current models of mantle velocity heterogeneity, discontinuity topography and radial viscosity structure strongly imply a whole-mantle pattern of circulation. This result seems to be robust with respect to considerable variations in these models, the velocity-density scaling relationship and the density jump across the 660-km discontinuity. But it should be possible to constrain our results better from improved seismic experiments and laboratory measurements in the near future. A critical challenge in future geodynamic modelling will be to find numerical models consistent with the observed pattern of mantle velocity and discontinuity perturbations. □

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Crystal structure of a yeast TBP/TATA-box complex

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The 2.5 Å crystal structure of a TATA-box complex with yeast TBP shows that the eight base pairs of the TATA box bind to the concave surface of TBP by bending towards the major groove with unprecedented severity. This produces a wide open, underwound, shallow minor groove which forms a primarily hydrophobic interface with the entire under-surface of the TBP saddle. The severe bend and a positive writhe radically alter the trajectory of the flanking B-form DNA.

WE report here the 2.5 Å crystal structure of the complex formed by the conserved carboxy-terminal domain of the yeast TATA-box binding protein (TBP) and a 12 base pair (bp) duplex containing the TATA box of the *CYC1* promoter. The formation of this complex is considered the nucleating event in the assembly of preinitiation complexes on eukaryotic RNA polymerase II promoters¹⁻³. As such, this structure provides a starting point for understanding the chemistry that defines the start site and polarity of transcription by RNA polymerase II.

TBP serves as a linchpin for the assembly of basal preinitiation complexes and, in association with a wide range of TBP-associated factors (TAFs), forms multi-protein complexes that interact with proteins that regulate transcription. The amino-terminal segment of full-length TBP varies in length and sequence depending on its cellular source and is dispensable for basal transcription and yeast cell viability⁴⁻⁶. By contrast, the 180 amino-acid C-terminal domain is highly conserved phylogenetically and exquisitely sensitive to mutations (Fig. 1). Its sequence has a striking repeat pattern with 28% of the residues in the first half of the molecule identical to those in the second half. Recent mutational and biochemical data have shown that TBP binds to the minor groove^{7,8}, and significantly bends the DNA⁹. The crystal structure of TBP-2 from *Arabidopsis thaliana*, solved by Burley and co-workers¹⁰, has given stereochemical context to the mutagenesis studies, and shows that the sequence repeat corresponds to a high degree of dyad symmetry. More recently, the crystal structure of the uncomplexed yeast TBP domain has also been solved and refined (ref. 11 and J.H.G., S.H., Y.K. and P.B.S., manuscript in preparation) enabling us to ascertain what, if any, structural changes occur on complex formation with the TATA box.

Structure determination and refinement

The 185 amino-acid conserved C-terminal domain of the yeast TBP (yTBPC) was cleaved from the full-length recombinant yTBP (overexpressed in bacteria) with endoproteinase Lys-C and purified to homogeneity (manuscript in preparation). Tetragonal crystals ($P4_3$, $a = 100.27$, $c = 65.99$ Å) were grown from a mixture of yTBPC and a 29-nucleotide hairpin construction (12 bp stem, 5 base loop) the stem of which encompasses the TATA box from the yeast *CYC1*(-52) promoter (Fig. 1). A hairpin was used to ensure stoichiometry of the duplex strands and to enhance duplex stability. These crystals contain two complexes per asymmetric unit and diffract well to 2.5 Å at -167 °C on laboratory sources and at least to 1.9 Å using synchrotron radiation. The structure was determined to 3.5 Å by multiple isomorphous replacement (MIR) using iodinated oligonucleotides. After phase improvement by solvent flattening¹², the elec-

tron-density map was good enough to model the DNA duplex and about 60% of the protein. This enabled us to establish the local symmetry operator and apply non-crystallographic symmetry averaging to improve the phases^{13,14}. Coordinates of TBP-2 from *Arabidopsis* (provided by S. Burley) enabled us to complete the interpretation. The model was refined to 3.2 Å against data collected at room temperature. The final model was refined to 2.5 Å with data collected at -167 °C from a much better ordered iodine-containing derivative. The current model includes two complexes, each containing 180 amino acids and a 29-base hairpin DNA construct, as well as a total of 298 water molecules. The final *R*-factor is 19.4% for all data (free *R*-factor = 22.1%) with excellent stereochemistry. Detailed procedures and statistics are given in Table 1, and the electron density is shown in Fig. 3.

Orientation and structure of the TATA box

The crystal structure of the complex defines the TATA box as an 8 bp element that contacts the protein throughout its length and deviates greatly from canonical B-form DNA (Fig. 2). The entire TATA box is severely bent toward the major groove exposing on its convex outer surface a very wide and shallow minor groove that interacts intimately with the entire concave under-surface of the TBP-saddle from stirrup to stirrup (Fig. 2). The orientation of the DNA is entirely different to that envisaged from examining the TBP structure alone^{10,11} in that it is bent and untwisted to an unprecedented degree with the DNA axis nearly perpendicular to the modelled one.

As the DNA arches under the saddle, its axis crosses the lateral axis of TBP (stirrup to stirrup) at a very shallow angle (~ 22 degrees) (Fig. 2). The TATA box is centred on the pseudodyad of the protein that passes between base pairs 4 and 5, and is oriented so that the beginning of the TATA box contacts the second direct repeat. The dyad divides the target element into two roughly symmetrical 4-bp segments. In response to the severe bend, the TATA box unwinds considerably (Fig. 4) enabling the DNA to maintain nearly symmetrical contact with the essentially flat under-surface of the TBP's saddle. This is reflected in an average helical twist angle of 18.5° , about half that of canonical B-form DNA. The greatest reduction in twist angle is at the very centre of the TATA box where the twist is only 3° . The unwinding of about 1/3 of a turn of DNA in 7 steps¹⁵ is compensated by a noticeable positive writhe (Fig. 4). The existence of this right-handed coil of the DNA axis, which compensates for the helical untwisting associated with TBP binding, was predicted on the basis of TBP's comparable affinity for TATA boxes in linear and closed circular constructs¹⁶.

Figure 4 shows the DNA conformational parameters. The curvature towards the major groove has a projected radius of 25 Å and is reflected in a consistently large positive roll angle

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for all steps within the element. This roll is especially large at the first and last steps (1→2 and 7→8) where values of 47° and 37° qualify them as 'kinks'. The roll is coupled to a severe buckling of base pairs 2 and, symmetrically, 7. The kink and buckle result from the wedging of two pairs of phenylalanine side chains into the minor groove, one pair between base pairs 1 and 2 and the other between 7 and 8. This severe deformation is stabilized by an intra-strand hydrogen bond between the first two nucleotides in the 'top' strand; that is between the O4 of T1 and the 6 amine of A2. T-A is a nearly invariant sequence at the 5' end of most TATA boxes and this unusual intra-strand interaction may help stabilize the deformation at the beginning of the TATA box. No other non-Watson-Crick hydrogen bonds occur between the bases.

Underwinding is reflected in short interphosphate distances with the sugar phosphate strand, here approaching the minimum value of 5.6 Å seen in A-form DNA^{17,18}. The deepening of the major groove to accommodate the bend is accompanied by a shallow and very wide minor groove (8.0–9.8 Å versus 4.2–6.7 Å

for crystalline uncomplexed B-form DNA) that provides a large accessible DNA surface with which to form a specific interface. The shortened interphosphate distance, the diminished helical twist and the wide and shallow minor groove reflect a tendency toward A-form DNA within the overall context of severe deformation^{17,18}. We defer conclusions about the role of the sugar pucker to a higher resolution refinement.

Remarkably, the helical parameters return abruptly to those of normal B-form DNA in the very first step outside the TATA box (0→1, 8→9). Thus, the deformation is restricted to and persists over the entire contact surface, producing a severe bend and untwisting that, when coupled to a compensatory writhe, radically alters the direction taken by the flanking B-DNA duplexes (Fig. 1c, d).

Structure of the protein

The structure of yTBPc is described in ref. 11 and in our manuscript in preparation and closely resembles that of *Arabidopsis*. Like the yTBPc/TATA-box complex, there are two crys-

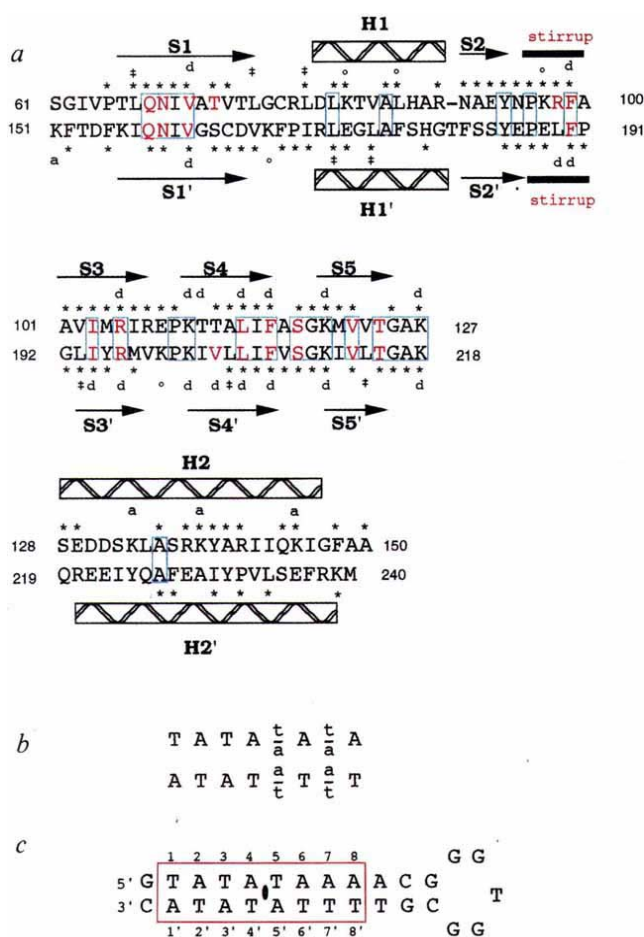


FIG. 1 Sequences and functional relevance of the components in the yTBPc/TATA box complex. **a**, Amino-acid sequence of yTBPc, as deduced from the encoding DNA³⁵ arranged as approximate direct repeats (first repeat, 61–150 above; second repeat, 151–240, below). Blue boxes include identical residues in both repeats; red indicates DNA contacts seen in the crystal structure; asterisks indicate phylogenetically conserved residues^{10,35,36}; °, points where mutations have no apparent effect on function²⁴; 'd', points at which mutations affect DNA affinity^{4,24,28,31,44}; 'a', points where mutations affect TFIIA binding^{26,27}; ‡, mutations affect both binding and transcription²⁴. **b**, Consensus TATA box. **c**, 29-nucleotide hairpin construction bearing the CYC1(52) TATA box used in the crystallization. The 5' end is at the left of the top strand; red box indicates the region contacted by yTBPc in the crystal structure. The num-

bering scheme is used throughout this article. **d**, Functional analysis of 29-nucleotide hairpin in which we measure binding of yTBPc to the 29-bp TATA oligonucleotide in **c**. Top, binding of yTBPc to the 29-bp oligonucleotide containing the CYC1 TATA (TATA_{HP}) and a 106-bp fragment containing the major late promoter sequence from –50 to +33 (TATA_{MLP}) assayed by electrophoretic mobility shift assay. The positions of unbound DNAs as well as TBP–DNA complexes are indicated. Bottom, plot of results in **a** as determined by PhosphorImager analysis (■) yTBPc-TATA_{MLP} (□) yTBPc-TATA_{HP}. Conditions for TBP binding and non-denaturing gel electrophoresis have been described⁴⁷. Binding reactions contained 1,000 c.p.m. of both TATA_{HP} and TATA_{MLP} fragments and was for 30 min at 25 °C.

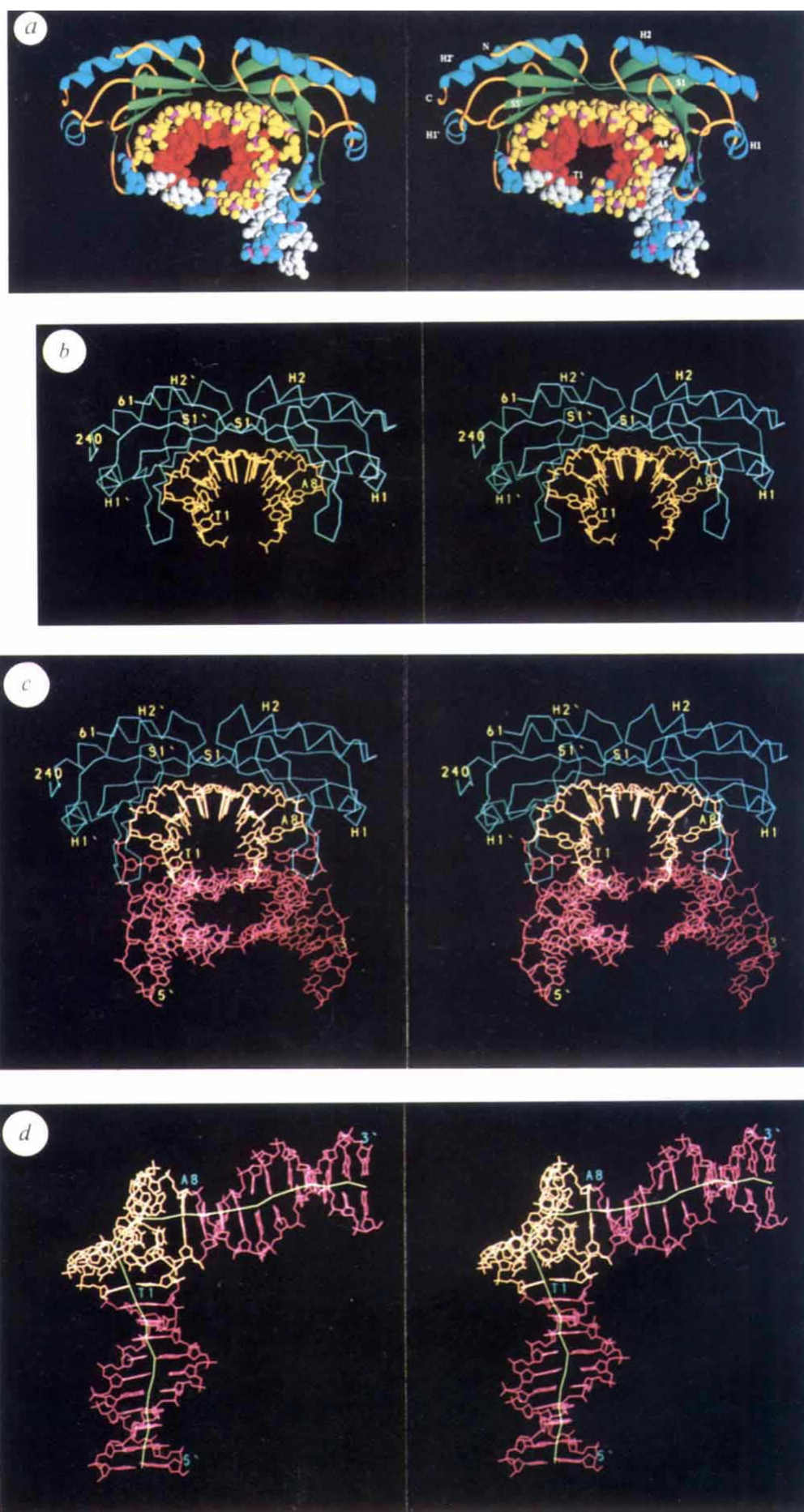


FIG. 2 The overall structure of yTBPC/TATA-box complex. *a*, Ribbon and space-filling drawing of the complex. The DNA is the hairpin construction with the loop to the right. *b–d*, Skeletal models of the TATA-box (in yellow); and, in *c–d*, extended in both directions with one turn of B-form DNA (in red). yTBPC is represented as a blue $C\alpha$ trace. The DNA axis (the bright green line) is the trace of the 'centre' of each base pair, that is, the midpoint of the line connecting the purine's C8 and the pyrimidine's C6. *b*, Frontal view (as in *a*) with the TATA box alone, starting on the left. *c*, The same view as in *a* and *b*, with B-form DNA extensions. *d*, View with 5' extension of DNA vertical and 3' extension in the plane of the paper, with yTBPC removed, to illustrate the effect of deformation on the trajectory of the DNA. The internal angle is $\sim 100^\circ$. Note the superhelical writhe in the TATA box itself.

TABLE 1 Summary of crystallographic analysis

(a) Phase determination									
Resolution limit (Å)	12.6	9.18	7.23	5.96	5.07	4.41	3.90	3.50	Total
IU69*									
Number of unique reflections	136	303	513	783	1,078	1,460	1,896	2,338	8,507
Phasing power	1.72	1.62	1.48	1.21	0.86	0.58	0.43	0.27	0.63
IU7†									
Number of reflections	127	261	444	684	948	1,291	1,651	2,047	7,453
Phasing power	2.21	2.04	1.83	1.78	1.34	0.90	0.67	0.47	0.93
Mean figure of merit	0.65	0.61	0.57	0.52	0.43	0.33	0.24	0.15	0.32
(b) Native data and refinement									
Resolution Limit (Å)	4.99	3.97	3.47	3.15	2.92	2.75	2.61	2.50	Total
Number of unique reflections	2,908	2,879	2,824	2,828	2,844	2,798	2,596	2,277	21,954
Predicted	2,929	2,884	2,836	2,847	2,857	2,839	2,814	2,828	22,834
$\langle I/\sigma \rangle$	26.0	26.6	23.6	18.6	11.8	7.54	5.15	3.24	19.0
R_{sym}	0.063	0.089	0.106	0.123	0.167	0.217	0.259	0.322	0.100
Number of reflections used in refinement (6–2.5 Å)	1,230	2,853	2,780	2,848	2,830	2,643	2,485	1,986	19,655
R-factor	0.175	0.138	0.157	0.188	0.228	0.268	0.283	0.311	0.194
Free R-factor									0.22
r.m.s. deviations		bond length 0.017 Å		bond angle 2.83°		dihedral angle 1.58°			

Recombinant yTBPC from overproducing *Escherichia coli* clones was purified as described (manuscript in preparation) and concentrated to 20 mg ml⁻¹ (1.0 mM). Oligonucleotides (29 nucleotides; 12 bp, 5 base loop) were synthesized by the phosphoramidite method and purified on a reversed-phase-C4 HPLC column (pH = 6.1, 0.1 M triethylamine-acetic acid and 15–25% acetonitrile gradient) at 50 °C followed by anionic exchange chromatography on a Mono Q (pH = 11.8, 0.01 M NaOH and 0.70–0.85 M NaCl gradient) at room temperature. DNA was annealed by heating at 90 °C for 5 min at low concentration (~0.05 mM) and chilling on ice. Iodinated DNA were prepared similarly, but in darkness to avoid photolysis. Purified DNA was concentrated to about 2 mM and mixed with yTBPC in a 2 to 1 molar ratio for crystallization. Crystals (0.1 × 0.1 × 0.5 mm) grew in 3 or 4 days at 22 °C from hanging drops (4 µl) containing 0.25 mM protein, 0.5 mM DNA, 500 mM NaCl or KCl, 20 mM 1,3-bis(tris-(hydroxymethyl)-methylamino) propane (BTP), pH 7.5, 15% PEG 8000, 3.75% glycerol and 1% ethylene glycol when equilibrated over a reservoir of 30% PEG 8000, 40 mM BTP pH 7.5, 2.5% glycerol, 2% ethylene glycol and 500 mM NaCl or KCl. These crystals diffracted to about 3.0 Å on a Siemen's Xenotronics area detector using double mirror-monochromated/focused Cu K α from a 0.2 mm focus of a Rigaku generator operating at 3 kW (50 kV × 60 mA). Initial native data and derivative data (IU69 and IU7) were collected at room temperature, processed by the program XDS^{37,38}, and scaled by SCALEPACK (Z. Otwinowski, Yale University). The iodine-containing 'parent' data used for the final refinement was collected to 2.5 Å on the Raxis II at -167 °C processed by DENZO (Z. Otwinowski) and scaled by SCALEPACK. Heavy-atom positions were determined from the difference Patterson maps and refined with ML-PHARE³⁹. MIR phases were improved by solvent flattening with ISIR/ISAS¹². The resulting electron-density map showed a clear trace for the β -strands and most of the DNA. Interpretation was helped considerably by referring to the model of *Arabidopsis* TBP, made available by S. Burley. Non-crystallographic symmetry averaging, using SQUASH^{13,14}, was also applied to the solvent-flattened map to improve the phases. X-PLOR⁴⁰ refinement with simulated annealing starting at 4,000 °K, gave an *R*-factor of 19.4% to 3.5 Å, but with unsatisfactory geometry for the DNA bases. Ten cycles of conventional restrained refinement with PROFFT^{41–43} restored proper geometry to the DNA with an *R*-factor of 22.1% to 3.2 Å. Using improved data from the iodine-containing 'parent' crystals, the refinement was extended with X-PLOR in stages to 3.0 Å, 2.6 Å and 2.5 Å and the model adjusted with the aid of appropriate difference maps (including 'omit' maps). Water molecules were added in the latter stages. Non-crystallographic symmetry was not imposed as a restraint and the free *R*-factor consistently converged. Except for glycines the Ramachandran plot shows no violations.

* IU69: (GTATATAAAACGGGTGGCGTU^UTTAU^U ATAC)/yTBPC, where U^U = 5-iodo-U.

† IU7: (GTATATAAAACGGGTGGCGTU^UTATATAC)/yTBPC.

‡ Native data (IU6; GTATATAAAACGGGTGGCGTU^UTTATATAC)/yTBPC.

$R_{\text{sym}} = \sum_i \langle |I_h - I_h| \rangle / \sum_i I_h$ where $\langle |I_h - I_h| \rangle$ is the average of the absolute deviation of a reflection I_h from the average I_h of its symmetry and Friedel equivalents.

Phasing power = $\sum |F_h^c| / [|F_p^c| \exp(i\Phi_c) + F_h^c] - |F_{ph}^c|$, $|F_h^c|$ = heavy-atom structure amplitude and $|F_p^c|$ and $|F_{ph}^c|$ are observed amplitudes for the protein and heavy-atom derivative, respectively, Φ_c is the calculated phase, and the sum is over all observations.

Figure of merit = $\int P(\Phi) \exp(i\Phi) d\Phi \int P(\Phi) d\Phi$ where P is the probability distribution of Φ , the phase angle.

tallographically independent representations in the asymmetric unit providing an estimate of the variance in structure due to differences in crystal packing and error in refinement. It has been pointed out that the backbone trace of the uncomplexed protein is essentially the same as that of the complexed protein (J.H.G., S.H., Y.K. and P.B.S., manuscript in preparation).

The interface

An extensive hydrophobic van der Waals surface. Figure 5a summarizes all the protein–DNA contacts. The interface is primarily hydrophobic. Of the 3,124 Å² of surface excluded from solvent by complex formation, only 1,080 Å² (34.5%) is hydrophilic. By contrast, most specific protein–DNA interactions have primarily hydrophilic interfaces; typical values are 57%, 59%, 50% and 52% for complexes of the *trp* repressor, CAP, E2 of papillomavirus and the glucocorticoid receptor, respectively^{19–22}. In addition to the minor groove edges of the bases, the sugar moieties provide 50% of the DNA's extensive van der Waals contact surface. Figure 5c shows how the average

plane of the sugar ring presents C1', C4', C5' and O4' to the non-polar interface. Curiously, the interface sequesters 12 of the 16 hydrogen-bond acceptors of the minor groove (N3 of adenine and O2 of thymine), without providing complementary hydrogen-bond donors; indeed, these polar groups are usually in van der Waals contact with non-polar amino-acid side chains. The 2-amino group of guanine and the 3-methyl group of 3-methyl-adenine sterically disrupt the interface^{7,8,23}.

Six specific hydrogen-bonding interactions between the protein and bases are symmetrical. The interface has only six hydrogen bonds involving the O2 of thymine and N3 of adenine. These occur at the very centre of the TATA box where the amide groups of two symmetrically disposed and well buttressed asparagine side chains (Asn 69 and Asn 159) donate a total of four hydrogen bonds, two each to adjacent bases on the same strand (Asn 69 to T4' and A5'; Asn 169 to A4 and T5) (Fig. 5e g). In addition, the N3's of A5' and A4 also accept hydrogen bonds from Thr 124 and Thr 215, respectively. This hydrogen-bonding scheme is probably suitable for all TATA-box

sequences because the O2 of thymine and N3 of adenine occupy nearly stereochemically equivalent positions, and therefore do not strongly discriminate between A·T and T·A base pairs.

Phenylalanine side chains penetrate between base pairs. The most noteworthy of the hydrophobic interactions are those that produce the kinks between the first two and last base pairs of the target. In both cases, the minor groove edges of the adjacent base pairs are prized apart and the interior base pair is severely buckled by a wedge of two conserved phenylalanine side chains which are mutationally implicated in DNA binding (Fig. 5*d, h*)^{4,31}.

Phosphates interact sparsely and asymmetrically. Of the 14 phosphates within the TATA box, only 6 are contacted directly or indirectly by the protein to form a total of 6 direct and 1 water-mediated hydrogen bonds. Although the contacted phosphates are symmetrically disposed around the pseudodyad, the stereochemistry of their interactions is not (Fig. 5). Note that all but one (Ser 118) are formed or supported by three arginine residues, the only arginine residues whose side chains are close to the phosphates. By contrast, there are seven lysine side chains close to the phosphates and none make direct or indirect hydrogen bonds to the phosphates, though six have been shown by mutagenesis to be necessary for DNA binding²⁴. Instead, the ζ -ammonium groups of these lysine residues are tied back through direct and water-mediated hydrogen bonds to acceptors within the protein. This suggests that the attractive electrostatic interaction may be mediated in part through a delocalized charge potential.

The major groove of the DNA is not contacted by TBPC, it is solvent exposed and highly hydrated. Although the major-groove edges are severely compressed into a central hub, the phosphates and major groove of the bound TATA-box as well as the flanking B-form DNA offers a potentially useful binding surface that could participate in the stereospecific assembly of the preinitiation complex.

TFIIA binding sites

TFIIA is a large ($\alpha\beta$)₂ tetramer in solution that binds strongly to the TBP/TATA-box complex and, in doing so, extends the 5'-end of the TBP footprint by about five bp^{2,25}. Although TFIIA binds to TBP alone, the interaction with the complex is much stronger, thus implicating DNA either directly or indirectly in the TFIIA interactions, a conclusion supported by the fact that lysine residues in the large subunit of TFIIA are necessary for

stable binding (S.H., unpublished result). Mutagenesis experiments^{26,27} have implicated residues in H2 of TBP in TFIIA binding which is about 22 Å from the nearest phosphate groups and 45 Å from those phosphates that are protected from DNase I in the extended footprint. These distances could be spanned by one protomer of TFIIA. Taken together the evidence suggests that in binding to the TBP/TATA-box complex, TFIIA recognizes the TBP/TATA-box complex as a structural unit, exploiting at the very least the negative electrostatic influence of the DNA phosphates; and possibly stereospecific direct contacts with elements of the bound TATA box or flanking DNA.

Physiological relevance of the structure

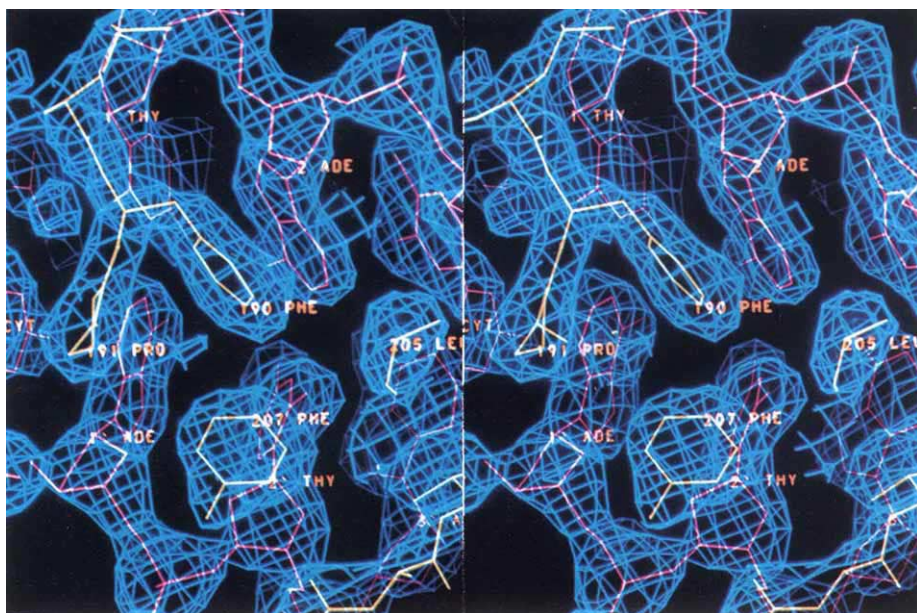
The unexpected and novel mode of interaction required that we address the evidence relating this crystal structure to the functional complex, particularly in view of the unusual DNA construction used in the co-crystallization. First, we have shown by gel shift analysis that the hairpin DNA used in this crystal structure binds to yTBPc with almost the same affinity as does the 106 bp duplex containing the major late promoter of adenovirus. Second, the structure is supported by essentially all of the available mutational evidence as discussed above (Fig. 1). Third, the complex is represented twice in the crystallographic asymmetric unit. Because these two complexes have different crystalline environments but virtually superimposable structures (legend to Fig. 5), the structure must be intrinsic to the chemistry of the complex and not imposed by lattice constraints.

The possibility of a rotationally inverted mode of binding, raised by the nearly symmetrical nature of the interface, is ruled out by the juxtaposition of a severely defective A2→G mutation and two amino-acid changes (Ile 194→Phe, Leu 205→Val) that combine to revert it²⁸. The model shows that the steric interference caused by introducing the 2-amine on the purine at the second position of the TATA box is relieved by the loss of a δ -methyl from the side chain of Leu 205. Although positioned close to the mutation, the stabilizing effect of replacing Ile with Phe at 194 is less clear.

Inverse curvature of the interface

Most sequence-specific DNA-binding regulatory proteins bind the major grooves of their target by presenting a convex surface that carries secondary structural elements designed to fit the major groove²⁹. As the major groove provides both better access and a wider variety of functional group distributions, it is an

FIG. 3 Electron-density map where phenylalanine side chains (190 and 207) penetrate the minor groove, prying apart the minor groove edges of the first two base pairs. Coefficients are $(2|F_o| - |F_c|) \exp(i\phi_c)$, where $|F_o|$, $|F_c|$ are the observed and calculated amplitudes, and ϕ_c is the phase calculated from the final refined coordinates. Contoured at 1.5σ .



Base number	0	1	2	3	4	5	6	7	8	9	10	11
Helical twist (°)	32.85	20.90	18.12	22.71	3.05	20.72	20.86	22.85	37.34	31.93	43.50	
Roll (°)		2.12	45.30	19.57	13.82	25.11	18.78	15.24	37.59	2.09	1.59	4.57
Slide (Å)		-1.61	-0.80	-0.91	1.63	0.79	1.67	0.40	0.51	-0.11	-1.23	0.07
Rise (Å)		3.47	5.49	3.71	3.22	3.78	3.59	3.74	5.32	3.35	3.50	3.65
P-P distance (Å)		5.63	6.27	5.50	5.75	5.80	6.24	5.75	6.70	7.04	6.58	7.04
Minor groove width (Å)		5.17	7.32	8.07	9.72	9.79	9.73	8.13	7.91	5.84	4.78	5.44
P-P distance (Å)		6.82	5.64	6.09	5.74	5.92	5.87	6.40	6.02	6.60	6.62	6.67
Buckle (°)		-10.10	-14.04	-38.77	-28.26	-5.55	11.03	23.02	24.49	6.67	12.35	5.20
Propeller twist (°)		-16.37	-13.27	5.05	-22.22	-10.32	-7.41	-24.83	-37.71	-0.37	-13.39	-15.80

FIG. 4 DNA helical parameters of the yTBPC/TATA-box calculated with CURVES³⁴. Minor groove width is defined¹⁵ as the shortest O4'-O4' distance minus 2.8 Å (two oxygen van der Waals radii). Standard values are 4.2–6.7 Å for regular B-form DNA and 3.8 Å for the Dickerson–Drew dodecamer⁴⁶.

effective architecture for DNA recognition. Less generally appreciated is a third virtue to this mode of interaction; namely, that to complement a convex protein surface the DNA often bends toward the major groove. The resulting compression of the concave surface causes the major grooves to deepen and become more narrow, thereby clamping down on the protein's identity elements with the bordering phosphates providing strong polar anchoring interactions.

In minor groove interactions, the minor groove cannot be compressed without further restricting access to the limited variety of the bases' functional contours. Indeed, to permit full access, it is advantageous for the minor groove to bend away from its recognition surface presenting a convex surface to the protein. This splay open the minor groove making it more shallow. To complement a convex minor groove surface, the protein's DNA-binding surface must be concave, an unusual situation seen in the extreme in TBPC. Thus, the TBPC/TATA-box complex presents a logical stereochemistry and, perhaps, a model for other specific minor groove interactions.

Specific affinity

What accounts for the stability of the TBP/TATA-box complex (dissociation constant, $K_d \sim 0.8-4 \times 10^{-9}$) (ref. 30) and TBP's 10^5 -fold preference for a TATA-box sequence over random DNA? The most obvious stabilizing feature of the interface is the large hydrophobic protein surface that blankets the minor groove including the sugar moieties as well as the edges of the bases (Fig. 5b, c). This surface, with the few exceptions discussed below, forms an intimate complementary van der Waals interface with a wide shallow minor groove that cannot tolerate the introduction by mutagenesis of polar or enlarged amino-acid side chains^{4,24,31} or the exocyclic 2-amine of guanine. This van der Waals surface is so all-encompassing that it buries most of the polar functional groups of the bases. It is punctuated only by a symmetrical set of polar contacts that fix the centre of the box (between base pairs 4 and 5) on TBP's pseudodyad.

The unusual degree of distortion suggests that the TATA box is recognized in part by 'indirect readout' of its sequence; that is, the TATA-box sequence permits the deformation required by this unique protein-DNA interface at a minimal cost in internal energy. It has been noted^{32,34} that alternating pyrimidine purine sequences, in particular -TATA- are especially flexible. Although A + T-rich, the 3' half of most TATA boxes do not have alternating A and T and are, therefore, likely to be less flexible than the 5' half. This asymmetry in deformability may provide part of the mechanism underlying the unique directionality with which TBP binds to the TATA element discussed below.

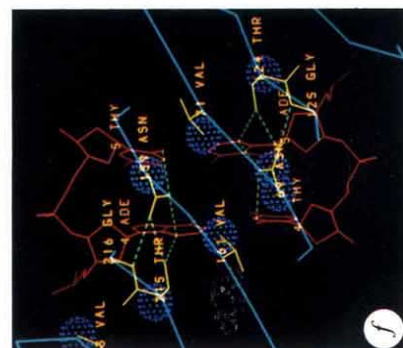
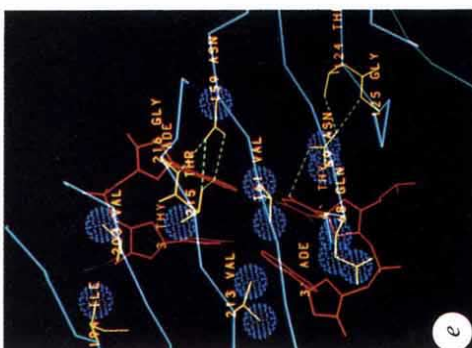
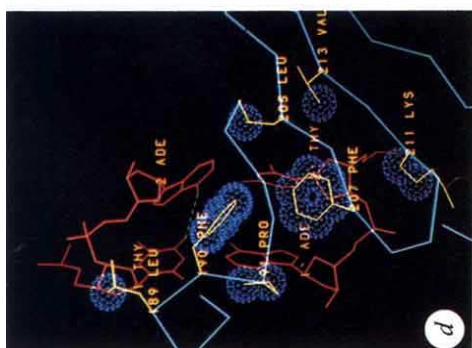
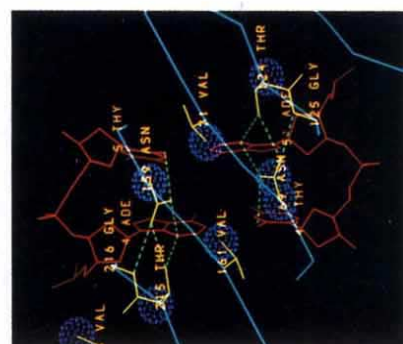
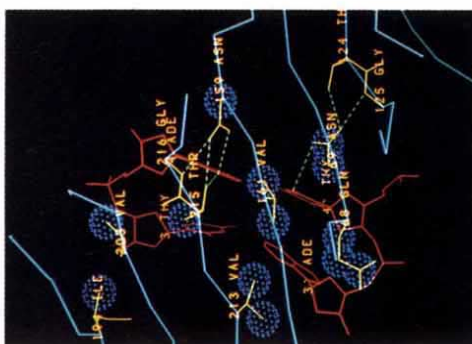
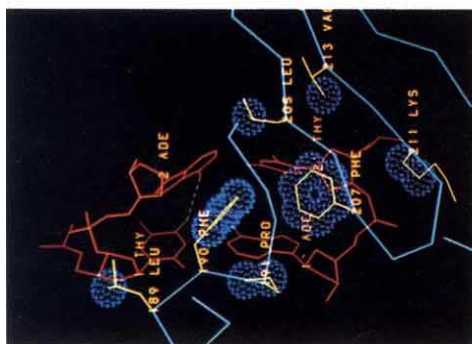
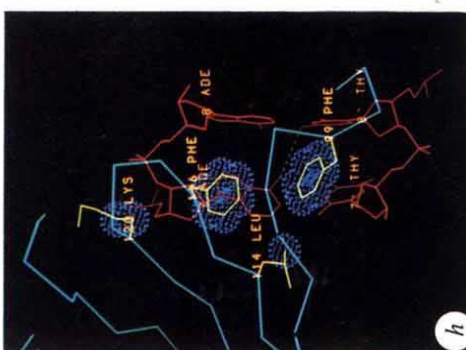
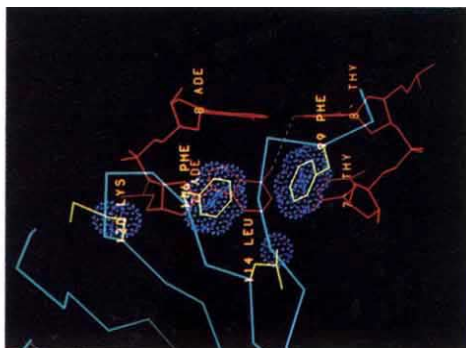
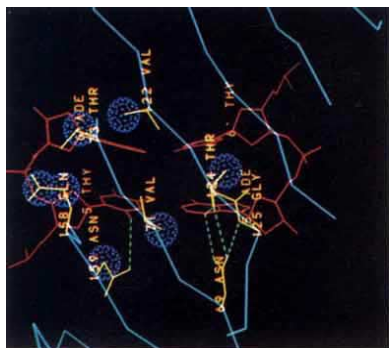
Effect on preinitiation assemblies

Bending of promoter DNA by proximal transcription factors is a common architectural theme helped by the flexibility of -TATA- promoter sequences or by a Py Pu step in specific binding sites³³. Proteins that deform DNA may help transcriptional initiation in several ways. One mechanism is to bend the DNA so as to bring the components of the preinitiation complex into proper apposition. Another mechanism invokes the fact that bending towards the major groove unwinds a duplex, thereby helping functions that require a single strand. Figure 2c and d shows the expected trajectory caused by the combination of severe bending towards the major groove, an associated untwisting of the duplex, and a compensatory positive writhe of the DNA axis. This unique trajectory sets the stereochemical context for transcriptional regulation by proteins that bind to the promoter and to proximal and distal regulatory elements. We view the TBPC/TATA-box complex and its immediate flanking DNA segments as a nucleoprotein structural unit whose functional groups and charge potential distribution determine the affinity and orientation by which the components of the preinitiation complex assemble and interact with regulatory factors. As there are no obvious conformational changes imposed on yTBPC by its interaction with the TATA box, it is not apparent that DNA-induced changes in yTBPC structure influence the subsequent assembly events. Finally, the fact that T-A-rich sequences prefer to wrap around histones with their minor grooves forming a concave surface facing inward³³ offers an attractive mechanism for the nucleosome's repressive effect on transcription. Whether or not inward facing TATA elements are effectively shielded from TBP by the nucleosome is being experimentally questioned (A. Imbalzano and R. Kingston, personal communication), however, it appears certain to us that when the TBP binds the TATA element, the inversion of curvature may well 'liberate' a significant segment of the promoter and help further assembly events.

Our model does not suggest that strand separation is an important consequence of TBPC binding to the TATA box. This follows from three observations. First, despite the extreme unwinding, Watson-Crick base pairing is maintained. Second, distortions of DNA end abruptly at the margins of the interface. Third, any strain induced by unwinding appears to be relieved by a compensatory writhe.

Polarity of assembly on the promoter

Generally the start site of transcription is 3' to the asymmetric consensus TATA-box sequence shown in Fig. 1. Moreover, the protein-binding surface of TBPC is asymmetric with distinct sites that contact various transcription factors. Therefore, to the



We conclude that with the stereochemical framework provided by this complex, we can better design and interpret experi-

ments that address the biochemistry of transcriptional initiation and its regulation. □

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Co-crystal structure of TBP recognizing the minor groove of a TATA element

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The three-dimensional structure of a TATA-box binding polypeptide complexed with the TATA element of the adenovirus major late promoter has been determined by X-ray crystallography at 2.25 Å resolution. Binding of the saddle-shaped protein induces a conformational change in the DNA, inducing sharp kinks at either end of the sequence TATAAAG. Between the kinks, the right-handed double helix is smoothly curved and partially unwound, presenting a widened minor groove to TBP's concave, antiparallel β -sheet. Side-chain/base interactions are restricted to the minor groove, and include hydrogen bonds, van der Waals contacts and phenylalanine–base stacking interactions.

THE TATA-box binding polypeptide (TBP or TFIID τ) is required by all three eukaryotic nuclear RNA polymerases (reviewed in refs 1–3). The mechanisms of action of TBP are best understood for transcription initiation of class II nuclear genes by RNA polymerase (pol) II with the general initiation factors TFIIA, -B, -D, -E, -F, -G/J, -H and -I (reviewed in refs 4, 5). In pol II transcription in higher organisms TBP is tightly associated with TBP-associated factors in a 700K multiprotein complex known as TFIID. This large complex recognizes the TATA element of class II nuclear gene promoters and thereby coordinates formation of a functional preinitiation complex with other class II initiation factors and pol II (reviewed in refs 6, 7). TBP cannot substitute for TFIID in assays of activator-dependent transcription^{8,9} (at least in higher eukaryotes), but can recognize the TATA element and mediate preinitiation

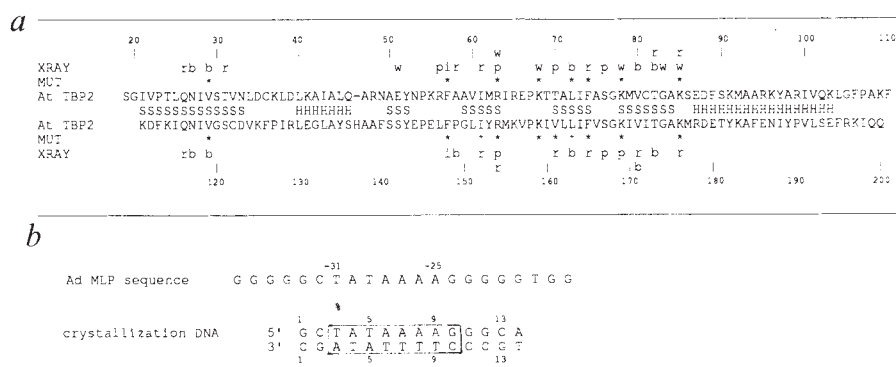
complex assembly¹⁰ and basal (core promoter) transcription initiation.

TBP has a phylogenetically conserved, 180 amino-acid carboxy-terminal portion (ranging from 38 to 93% identity), containing two structural repeats flanking a highly basic segment known as the basic repeat (reviewed in ref. 11). The C-terminal or core portion of the protein binds the TATA consensus sequence (TATAa/tAa/t)^{12,13} with high affinity ($K_d = 2\text{--}4 \times 10^{-9}$ M)¹⁴, interacting with the minor groove^{15,16} and promoting DNA bending¹⁷. The amino-terminal portion of TBP varies in length, shows little or no sequence conservation among different organisms, and is largely unnecessary for transcription in certain yeast strains (reviewed in refs 18, 19).

Recently, we reported the X-ray crystal structure of TBP isoform 2 (TBP2) from *Arabidopsis thaliana*¹¹, which displays a novel, two-domain DNA-binding fold. The protein's approximate 2-fold intramolecular symmetry generates a saddle-shaped

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FIG. 1 a, Amino-acid sequence of TBP2 from *A. thaliana* with the two structural repeats aligned one above the other. Residues were assigned to secondary structural elements according to criteria defined in ref. 52. Residues in α -helices and β -strands are denoted with H and S, respectively, and the TBP2 numbering scheme is used throughout (*Saccharomyces cerevisiae* TBP numbering corresponds to TBP2 numbering plus 42). The locations of point mutants and their phenotype are indicated as Mut: *, DNA binding and +, DNA recognition. The locations of residues in the X-ray co-crystal structure making DNA contacts are denoted by XRAY: p, phosphate; w, water-mediated phosphate; r, ribose; b, base; and i, intercalating phenylalanine residues. **b**, Partial sequence of the AdMLP²⁰ aligned with the sequence of the duplex oligonucleotide used in co-crystallization. The numbering scheme of the AdMLP is used throughout the text, and the locations of the two iodine-labelled bases



used for the structure determination are denoted by %. A box encloses the eight base pairs involved in TBP-DNA recognition.

structure dominated by a curved antiparallel β -sheet, forming a concave DNA-binding surface. The seat of the saddle presents a large convex surface to which other proteins bind during transcription initiation. The structure of TBP2 demonstrated that it could bind both DNA and a myriad of other transcription factors, but did not explain specific recognition of pol II TATA elements.

We now report the co-crystal structure of a canonical TATA-box binding protein recognizing the TATA element of the adenovirus major late promoter²⁰ (AdMLP). The three-dimensional structure of full-length TBP2 from *A. thaliana* complexed with its cognate DNA, determined at 2.25 Å resolution, reveals that the saddle-shaped molecule undergoes a conformational change on binding to the minor groove of a dramatically distorted, partially unwound double helix. This structure clarifies the

nature of the unprecedented interactions that permit TBP to recognize DNA.

Structure determination and refinement

TBP2 from *A. thaliana* was expressed, purified and co-crystallized with a 14 base pair (bp) oligonucleotide (Fig. 1, Table 1). Quantitative electrophoretic mobility shift assays documented specific, nanomolar-affinity binding of the purified, recombinant protein to the co-crystallization DNA¹¹. The monoclinic co-crystals ($P2_1$; $a = 41.8$ Å, $b = 146.5$ Å, $c = 57.6$ Å, $\beta = 90.6^\circ$) diffract isotropically to 1.9 Å. The asymmetric unit includes two DNA-protein complexes.

The structure was initially determined at 2.8 Å resolution by a combination of molecular replacement and multiple isomorphous replacement (Fig. 1b, Table 1), followed by phase extension

FIG. 2 Electron density of portions of the TBP2-DNA complex, depicting distorted features of the DNA. The refined structure of the protein and DNA are illustrated as atomic stick figures, using colour coding for atom type (C, yellow; O, red; N, blue; S, green). **a**, Stereodrawing of a simulated annealing omit ($2|F_{\text{obs}}| - |F_{\text{calc}}|$) difference Fourier synthesis contoured at 1.2σ . Base pairs -30 and -31 and amino acids Phe 148 and Phe 165 were omitted from the X-ray model and simulated annealing refinement was done with a 3 Å spherical shell of fixed atoms surrounding the omitted region (maximum temperature = 1,000 K, resolution limit = 2.25 Å). The 40° roll between base pairs T-A(-31) and A-T(-30) is clearly visible, with Phe 148 packing against the base of A(-30) and Phe 165 packing against the ribose of T(-30). **b**, Stereodrawing of a ($2|F_{\text{obs}}| - |F_{\text{calc}}|$) difference Fourier synthesis showing the protein-DNA interface in the middle of the TATA element (contoured at 1.6σ). Close packing of side chains and the minor groove edges of the bases can be readily appreciated.

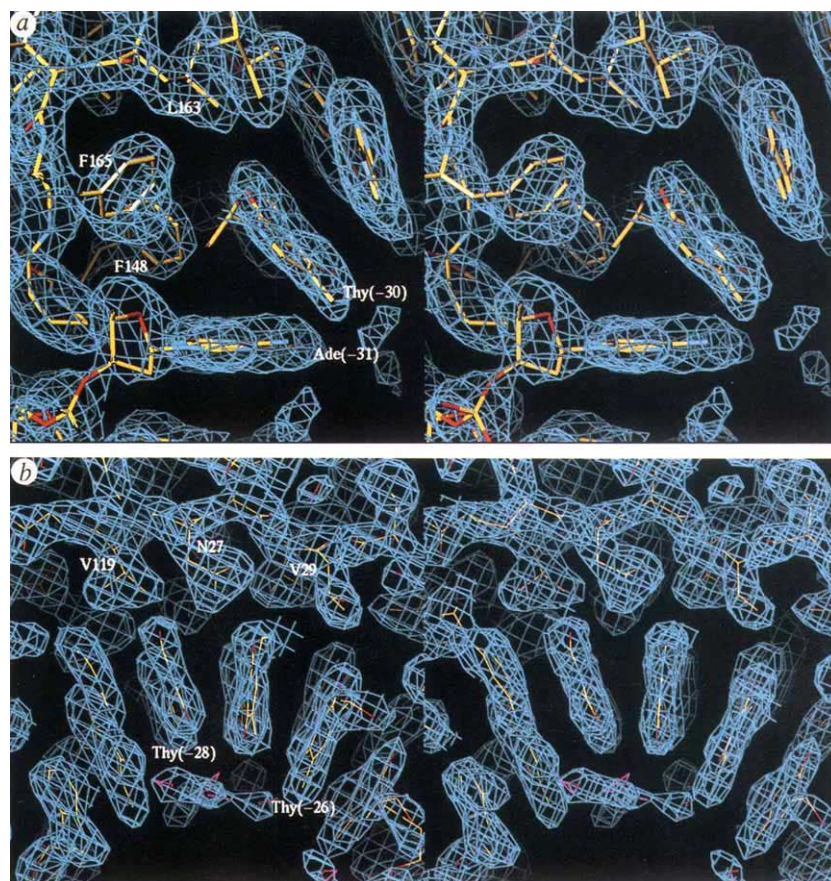


TABLE 1 Summary of crystallographic analysis

	Native 1		Native 2		Iodo-dU9		Iodo-dU3	
Resolution (Å)	2.60		2.25		2.80		2.70	
Reflections (unique/observed)	18,312/47,659		29,632/153,868		14,092/33,054		16,440/55,094	
Data coverage (%)	87		90		83		87	
R_{sym} (%)	6.0		7.4		9.3		12.7	
MIR analysis (15–2.8 Å versus native 1)								
Mean fractional isomorphous difference (%)					15.8		21.8	
Number of sites					2		2	
Phasing power					1.03		0.93	
R_c					0.64		0.75	
Mean overall figure of merit	0.39							
Phase combined figure of merit	0.57							
Refinement statistics (native 2)								
Resolution (Å)	6.0–2.25	3.78	3.14	2.79	2.55	2.38	2.25	
R -factor (%)	20.2	15.9	17.9	22.4	25.4	28.6	30.8	
Reflections ($ F > 1.5\sigma F $)	27,613	5,149	5,129	5,071	5,062	4,348	2,854	
% Complete	89.3	99.7	99.2	98.8	98.0	84.9	55.4	
Number of non-hydrogen atoms	4254							
$r.m.s.$ bond length (Å)	0.014							
$r.m.s.$ bond angle (degrees)	1.97							
$r.m.s.$ B-factor bonded atoms (Å ²)	2.59							

TBP2 was purified as described previously¹¹. DNA was synthesized using standard phosphoramidite chemistry, and purified by anion exchange chromatography at pH 10.0 and 55 °C. Single-stranded DNA was quantified by ultraviolet spectrophotometry using extinction coefficients calculated incorporating nearest-neighbour contributions⁵³, mixed with an equimolar amount of complementary strand, and annealed by heating to 94 °C for 10 min and then cooling to 4 °C at a uniform rate of 0.5 °C min⁻¹. TBP2–DNA complex was prepared to a final concentration of 0.5 mM in 100 mM KCl, 250 mM ammonium acetate, 4 mM MgCl₂, 15 mM dithiothreitol, 14% (v/v) glycerol, 50 mM MES, pH 6.2. The DNA–protein complex was relatively insoluble under low salt conditions, enabling crystals to be grown at 4 °C by vapour diffusion against a reservoir containing 14% (v/v) glycerol, 15 mM dithiothreitol, 25 mM MES pH 6.2. Following macroseeding, chunky plates grew over the course of days with typical dimensions of 0.6 × 0.4 × 0.15 mm³. Crystals of heavy-atom derivatives were prepared in the same manner using DNA in which 5-iodoU had been substituted for T in the positions denoted in Fig. 1b. During the anion exchange purification step dehalogenated DNA was removed using the pK_a difference between bases with and without the halogen. Diffraction measurements were done at –150 °C using a Rigaku RAXIS-IIc imaging plate area detector and a refrigerated nitrogen gas delivery system. Crystals were transiently cryoprotected in a 27% glycerol, 9% PEG4K, 10 mM MgCl₂ mixture before freezing on a platform created by a loop of ophthalmological suture material (10-0 Ethilon, Ethicon). Despite isolation of the crystal and area detector in a nitrogen-filled chamber and frequent dusting of the crystal, modest accumulations of frost on the frozen crystal and supporting loop produced an X-ray powder line of ice at about 3.8 Å resolution, reducing heavy-atom derivative phasing power in the resolution shell between 4.0 and 3.6 Å resolution to below 1.0. Oscillation photographs were integrated, scaled and merged using DENZO and SCALEPACK (Z. Otwinowski, personal communication). Molecular replacement (MR) was done with X-PLOR²² using the structure of uncomplexed TBP2¹¹. Patterson correlation refinement of the rotation function search maxima yielded two distinct solutions with correlation coefficients of 0.099 and 0.094, respectively. Two independent translation searches in the X–Z plane followed by a relative Y-translation search, gave a final MR solution 6.1σ above the mean peak level. Rigid body refinement of the four domains of the two polypeptide chains in the asymmetric unit, followed by positional and simulated annealing refinement with X-PLOR gave an R -factor = 0.345 at 2.8 Å resolution. Although the electron density for the protein was well defined in $(2|F_{\text{observed}}| - |F_{\text{calculated}}|)$ difference Fourier syntheses, there was no interpretable electron density for the DNA, necessitating X-ray diffraction data collection from two iodinated-DNA co-crystals (Fig. 1b). Iodine atoms were located by isomorphous difference Patterson analyses, and confirmed by isomorphous difference Fourier syntheses using phases from the refined MR solution. Refinement of the heavy-atom model against both isomorphous and anomalous differences gave a final figure of merit (f.o.m.) of 0.39 (ref. 54). Phase combination of the MIR data with the refined MR model⁵⁵ increased the f.o.m. to 0.57, revealing continuous electron density for both the protein and the nucleic acid. Knowledge of the iodine atomic positions also helped to fix the registration and orientation of the DNA in the complex. Model building²² and phase combination interspersed with positional refinement allowed an unambiguous trace and sequence assignment of the four DNA strands. Additional crystallographic software included the PROTSYS package (G. A. Petsko, personal communication), CURVES²⁸ and COMBINE⁵⁵. Atomic coordinates and structure factor amplitudes have been submitted to the Brookhaven Protein Data Bank⁵⁶. $R_{\text{sym}} = \sum |I - \langle I \rangle| / \sum I$, where I = observed intensity, $\langle I \rangle$ = average intensity obtained from multiple observations of symmetry related reflections. Mean fractional isomorphous difference = $\sum |F_{\text{PH}}| - |F_{\text{P}}| / \sum |F_{\text{P}}|$, $|F_{\text{P}}|$ = protein structure factor amplitude, $|F_{\text{PH}}|$ = heavy-atom derivative structure factor amplitude. Phasing power = r.m.s. ($|F_{\text{H}}|/E$), $|F_{\text{H}}|$ = heavy atom factor amplitude and E = residual lack of closure. R.m.s. bond lengths and r.m.s. bond angles are the respective root-mean-square deviations from ideal values. R.m.s. thermal parameter is the root-mean-square deviation between the B values of covalently bonded atomic pairs.

to 2.25 Å resolution with model building²¹ and further refinement²². Subsequently, well ordered solvent molecules were included, and tightly restrained individual isotropic B-factors refined. The current model consists of two copies of the protein–DNA complex (14-bp oligonucleotide and residues 13–198 of TBP2) and 199 water molecules. Refinement statistics are given in Table 1. The electron density for the polypeptide backbone is continuous everywhere at 1.2σ in a $(2|F_{\text{observed}}| - |F_{\text{calculated}}|)$ difference Fourier synthesis, except for breaks in the loops connecting β-strand S1 and α-helix H1, and α-helix H1 and β-strand S2, respectively, in both monomers (Fig. 2 shows representative views of the final electron density). A Ramachandran analysis²³ revealed 4/370 unfavourable backbone torsion angle combinations (not shown). The DNA electron density is well defined throughout.

Structure description

Overview. The TBP2–DNA complex is illustrated in Fig. 3. DNA binds to the underside of the molecular saddle, but TBP does not simply straddle B-form DNA, as model building had suggested was possible¹¹. Instead, the oligonucleotide is greatly distorted. The 5' end of the DNA is B-form, entering the concave surface of the molecular saddle near the C-terminal stirrup. Between the first two base pairs of the sequence TATAAAAG there is a sharp kink in the DNA. A comparable kink occurs at

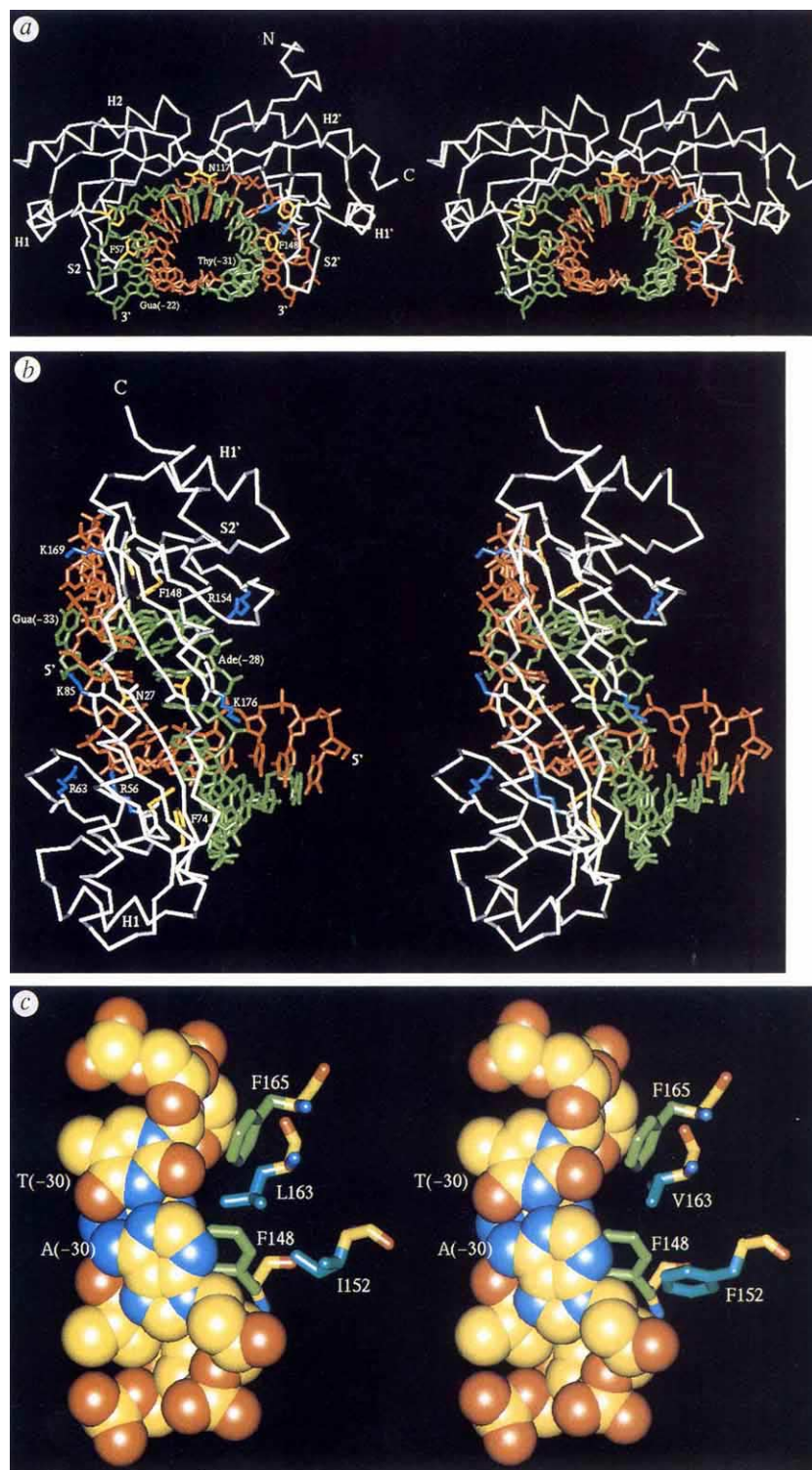
the A·T·G·C step. The 3' end of the oligonucleotide exits from beneath the molecular saddle as B-form DNA at an angle of 100° with respect to its direction of entry. Between the kinks, the right-handed, double-stranded DNA is smoothly curved and partially unwound, presenting the minor groove face of the sequence TATAAAAG to the antiparallel β-sheet of TBP.

Non-crystallographic symmetry. The asymmetric unit consists of two crystallographically independent, structurally similar complexes (root mean square (r.m.s.) deviations between α-carbon positions = 0.45 Å; r.m.s. deviations between phosphorus positions = 0.48 Å for the sequence TATAAAAG and 1.28 Å for the entire oligonucleotide). The two complexes in the asymmetric unit are related by the 2-fold rotation and translation, and make unique contacts within the crystal lattice.

Protein conformational change. TBP2 undergoes a modest conformational change on DNA binding, allowing it to follow the minor groove of the distorted oligonucleotide. Each domain is largely unaffected by DNA binding (r.m.s. deviations for α-carbon positions between each of the complexed and uncomplexed domains = 0.52–0.54 Å). Structural differences between complexed and uncomplexed TBP2 arise primarily from a 10° twist of one domain with respect to the other, shifting the relative positions of the two stirrup-like loops by 5 Å.

Unusual DNA conformation. Figures 3, 4 and 5 illustrate the dramatically distorted structure of the TBP2-bound oligonucleo-

FIG. 3 Structure of the TBP2-DNA complex. *a*, Stereodrawing viewed at 90° to the axis of roughly intramolecular 2-fold symmetry, showing the -31 end of the AdMLP sequence entering the underside of the molecular saddle from below. The 3' end of the oligonucleotide exits the saddle as B-form DNA with its cylindrical axis nearly perpendicular to the page (the last two base pairs were omitted for clarity). The two pairs of phenylalanine side chains inducing sharp kinks in the DNA are coloured yellow (Phe 57, Phe 74, Phe 148 and Phe 165). Between the two kinks the DNA is partially unwound. Two hydrophobic residues implicated in specific DNA recognition are coloured blue (Ile 152 and Leu 163)³⁴. The two symmetrically placed asparagine residues that make hydrogen bonds in the minor groove of the TATA element are also coloured yellow (Asn 27 and Asn 117). The remainder of the protein is depicted as a white α -carbon trace and the DNA is drawn as an atomic stick figure. Secondary structural elements are labelled according to ref. 11. The coding and non-coding strands of the DNA are coloured green and red, respectively. *b*, Stereodrawing viewed parallel to the axis of approximate intramolecular 2-fold symmetry, showing the molecular saddle following the widened minor groove of the partially unwound, double-stranded DNA ladder. Positively charged residues making salt bridges with the phosphodiester backbone are depicted as blue side chains. *c*, Drawing of the T:A:A-T step (-31 to -30 of AdMLP) and surrounding residues. The DNA van der Waals surface is colour coded for the underlying atom type (C, yellow; O, red; N, blue; P, yellow). Nearby protein residues are drawn as stick figures. Phe 148 and Phe 165 side chains are coloured green. The left panel depicts wild-type residues Ile 152 and Leu 163, with cyan-coloured side chains. In the right panel these residues have been replaced by Phe and Val, respectively, to show the effect of the specificity-broadening mutations that permit a mutant form of TBP to recognize both TATA- and TGTA-containing elements³⁴.



tide. The right-handed double helix makes standard Watson-Crick base pairs throughout, with bases in the *anti* conformation. Sugar puckers were restrained towards C2'-endo during crystallographic refinement, but they show no clear preference for any pucker at this resolution limit.

Selected analyses of the DNA conformation are presented in Fig. 4*b*. Throughout the oligonucleotide, shift, slide and tilt values show minimal variation about zero (not shown). The first three and last five base pairs are essentially B-form (average nucleotide parameters of rise = 3.3 Å, roll = 6.3°, twist = 31.7° or 11.3 bp per turn). The intervening six base pairs (ATAAAA)

demonstrate a novel, partially unwound, right-handed double helix (for a review of DNA bending see ref. 24). At the 5' end of the TATA element where the first kink occurs, the T·A(-31):A·T(-30) step has rise = 5.3 Å, roll = 40° and twist = 18° (rise is distance along the local helix axis between successive base pairs, roll is angle between the planes defined by two successive base pairs, twist is angular twist of the double helix between successive base pairs). A similar situation prevails at the second kink, where the A·T(-25):G·C(-24) step has rise = 5.7 Å, roll = 47° and twist = 26°. The 6-bp segment includes a short A-tract^{25,27}, and bends continuously towards the major

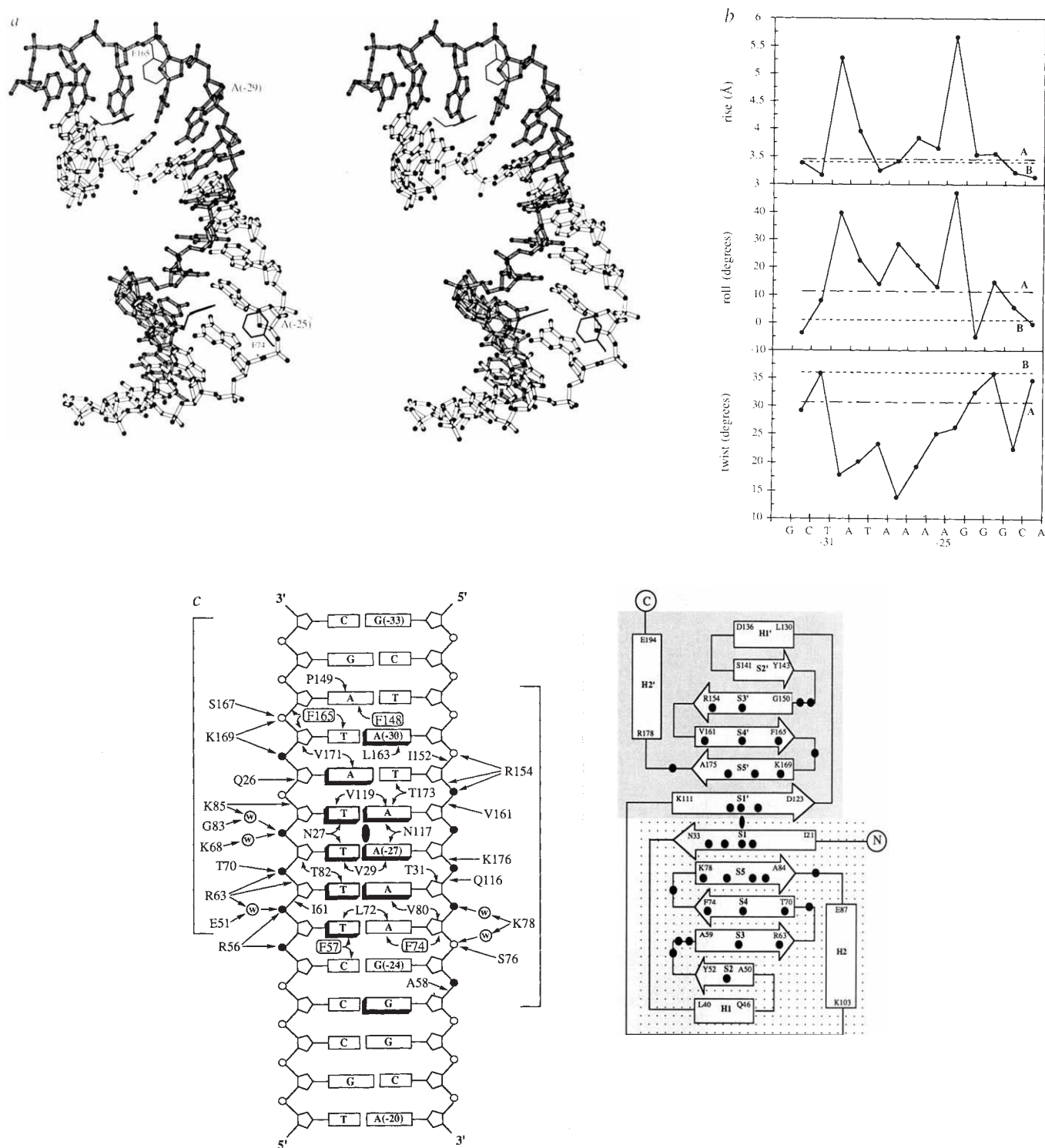


FIG. 4 Structural features of DNA binding. *a*, Stereodrawing of the atomic stick figure of the 14-bp oligonucleotide with the structure of the protein removed except for the side chains of the four phenylalanine residues involved in DNA kinking. The non-coding strand is indicated with shaded bonds. This view is roughly 90° from that shown in Fig. 3*b*. *b*, Variation of rise distance, and roll and twist angles for the oligonucleotide complexed with TBP2 (see ref. 28 for definitions of DNA structural parameters, which are calculated relative to a local helix axis). Values for A- and B-form DNA are indicated. *c*, Schematic views of the TBP2-DNA interaction. The left panel shows the protein contacts with the 14-bp oligonucleotide. The results of the chemical protection

studies of Lee *et al.*¹⁵ are also shown for comparison. Free radical footprints are indicated by brackets. Phosphate groups protected from ethylation by yeast TBP binding are coloured black. Bases implicated in protein-DNA interactions as judged by the results of treatment with dimethylsulphoxide (purines), or KMnO_4 or hydrazine (thymines) are outlined in bold. The right panel shows the secondary structure of TBP2 with the residues making protein-DNA contacts indicated with black dots. The β -strands are oriented to correspond to the view in Fig. 3*b*. In both panels the location of the approximate 2-fold axis of intramolecular symmetry is denoted by $\bullet$.

groove with average nucleotide parameters of rise = 3.6 Å, roll = 20° and twist = 20° or 18 bp per turn. At first glance this segment resembles A-form DNA, but the two conformations differ in slide, twist and roll values (for A-DNA slide = 1.9 Å, twist = 30.7° and roll = 11.4°)²⁸. The large negative propeller angles (propeller = -20.6°) and large positive roll angles within the short A-tract produce a series of bifurcated hydrogen bonds, as seen in crystal structure of two straight A-tracts^{26,27}. The ATAAAA segment also demonstrates systematic variation in buckle angle, resulting from insertion by TBP2 of phenylalanines into the flanking kinks (not shown). Finally, reduced twist and large positive roll angles considerably broaden the minor groove, ranging in width from 7.4 to 9.9 Å (defined as the distance between closest O4' atoms minus 2.8 Å to correct for the van der Waals radii of the two oxygen atoms; B- and A-form DNA values are 4.0 and 6.2 Å, respectively).

These distortions direct the double helix through two approximately 90° turns, producing a lateral displacement of 18 Å and a 100° angle between the cylindrical axes of incoming and outgoing B-form double helices (Fig. 4a, and 5). It appears, therefore, that partial unwinding of the TATA element has been compensated for by introduction of about one half a turn of a right-handed superhelix (consistent with studies of TBP binding closed circular DNA²⁹). Thus the unwound DNA presents its widened minor groove to the concave β -sheet of TBP.

Minor groove binding

TBP2-DNA contacts are completely restricted to the phosphate-ribose backbone and the minor groove (Fig. 1a, 2, 3 and 4c). Although their crystal lattice environments are not the same, the two crystallographically independent complexes contain essentially identical protein-DNA interactions. Both DNA strands and all eight antiparallel β -strands lining the underside of the molecular saddle plus their connections participate in complex stabilization. Like TBP2 and the TATA element themselves, the pattern of side-chain/DNA interactions has roughly 2-fold symmetry (Fig. 4c). Complex formation buries large portions of both the protein and DNA surfaces (total buried area $\approx 3,000$ Å²), and there is no evidence of water molecules between the protein and the DNA bases. With one exception, every residue for TBP implicated in DNA binding by site-directed mutagenesis (reviewed in ref. 11) participates in TBP2-DNA contacts (Fig. 1a). The exception, Lys 159, makes a salt bridge with a neighbour in the crystal lattice. Finally, all residues making DNA contacts are conserved throughout known TBP sequences (reviewed in ref. 11).

Side-chain/nucleic acid backbone interactions include salt bridges, water-mediated hydrogen bonds, and van der Waals contacts with ribose groups (Figs 2b, 3b, 3c and 4c). These enthalpically favourable interactions stabilize the protein-DNA complex and the unusual DNA conformation therein.

Interactions with the minor groove can be divided into three classes. (1) At either end of the TATA element there are two pairs of phenylalanine side chains partially inserted between adjacent base pairs, producing the two dramatic kinks (Figs 2a, 2c and 4a). Between T·A(-31) and A·T(-30), Phe 148 is packed against the A(-30) base and Phe 165 packs against the A(-31) ribose while making van der Waals contact with Phe 148, causing significant rise and roll at this base-pair step. Phe 57 and Phe 74 have a similar effect on the A·T(-25):G·C(-24) step at the 3' end of the sequence TATAAAAG, with Phe 57 packing against the base of T(-25) and Phe 74 packing against the ribose of A(-25) and also making van der Waals contact with Phe 57. Base stacking interactions at T·A:A·T and A·T:G·C base-pair steps are among the least enthalpically favourable of the 10 possible base-pair stacking combinations³⁰, minimizing the energetic penalty of introducing the two kinks in the TATA element. The kinks are themselves stabilized by the observed phenylalanine-base π - π interactions. (2) Four polar side chains make minor groove hydrogen bonds with acceptors of four base pairs centred

about the approximate 2-fold symmetry axis (Figs 3b and 4c). Side-chain/base hydrogen bonds occur between Asn 27 and T(-28) (N δ -O2 = 3.2 Å) and T(-27) (N δ -O2 = 2.9 Å), and between Asn 117 and A(-28) (N δ -N3 = 3.2 Å) and A(-27) (N δ -N3 = 3.2 Å). One base pair removed on either side, Thr 82 interacts with T(-26) (O γ -O2 = 3.8 Å) and Thr 173 interacts with T(-29) (O γ -O2 = 3.4 Å). (3) Fifteen residues projecting from the concave surface of TBP make hydrophobic or van der Waals side-chain/base contacts (<4 Å between non-hydrogen atoms) with the 8-base-pair sequence (TATAAAAG) flanking the approximate 2-fold axis (Fig. 4c). The remainder of the amino acids projecting from the β -sheet do not appear to make minor groove contacts, although other close contacts cannot be completely ruled out at this stage in the crystallographic analysis.

Complete absence of interactions between TBP2 and the major groove confirms earlier predictions that TBP binds to the minor groove of the TATA box^{15,16}. Our co-crystal structure compares favourably with results from chemical modification and footprinting studies of yeast TBP interacting with the AdMLP¹⁵ (Fig. 4c). The hydroxyl radical footprint of yeast TBP corresponds to the surface of our co-crystallization oligonucleotide buried in the complex. Phosphate groups protected from ethylation by yeast TBP are similarly solvent inaccessible. With the sole exception of G(-24), all of the bases implicated in DNA binding by the results of base modification within the TATA element of the AdMLP are involved in side-chain-base contacts in the co-crystal structure.

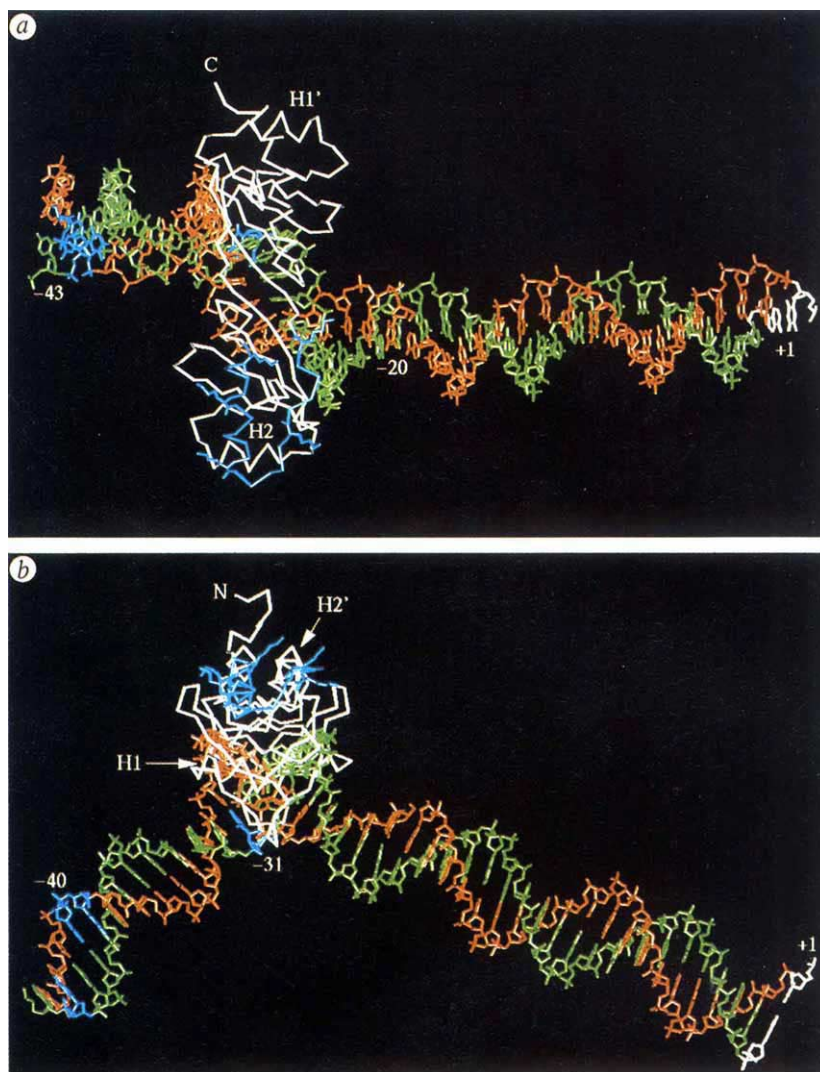
TATA element recognition

The structure of the TBP2-DNA complex represents the first example of a protein that binds DNA in a sequence-specific manner without recourse to side-chain/major groove contacts (reviewed in refs 31, 32). Moreover, it does so by bending an A+T-rich sequence towards the major groove, which directly refutes earlier predictions¹⁷. How, then, does TBP recognize a variety of TATA elements before directing preinitiation complex assembly in pol II transcription initiation? Side-chain/minor groove interactions and deformability of the DNA and, to a lesser extent, the protein are all implicated in the process.

Studies of pol II transcription initiation from TATA elements with single base-pair mutations provide useful insights into the importance of individual side-chain/minor groove contacts³³. No single base pair within the AdMLP TATA element is absolutely required for *in vitro* transcription initiation, but certain substitutions reduce yeast TBP activity to 5% or less (T·A(-31)→G·C, A·T(-30)→C·G or G·C, T·A(-29)→A·T or G·C, A·T(-28)→C·G or G·C, A·T(-27)→C·G). Each of these severe AdMLP TATA box mutations corresponds to a position in the complex where one or both of the bases is less than 4 Å from a hydrophobic side chain. For example, the deleterious effects of A·T(-30)→C·G or G·C can be explained by the presence of Leu 163 in the minor groove adjacent to A(-30) C δ 1-C2 = 3.6 Å), where it would make an unfavourable contact with the exocyclic NH₂ of a G. Similar close contacts between Val 171 and A(-29), Val 119 and A·T(-28), Val 29 and A·T(-27), and Val 80 and A(-26) explain the effects of the remaining severe AdMLP TATA element mutations. The importance of van der Waals contacts in TBP-DNA recognition is also emphasized by recent characterizations of DNA-binding specificity mutants of yeast TBP^{34,35}. For example, a double point mutant (Ile 152→Phe, Leu 163→Val) permits yeast TBP recognition of both TATA- and TGTA-containing elements. Figure 3c demonstrates that this double point mutation would abolish the van der Waals interaction between C δ 1 of Leu 163 and C2 of A(-30), creating a cavity within the tightly packed TBP-DNA interface that will accommodate the NH₂ of G in this position.

Severe TATA box mutations can be explained by the presence of two pairs of valines and one pair of leucines on the protein's DNA-binding surface, but these side-chain pairs are related by

FIG. 5 Schematic drawings of TBP2 interacting with the AdMLP. The DNA is depicted as a stick figure, with hypothetical, linear B-form DNA extensions added to both the 5' and 3' ends of the oligonucleotide structure determined using X-ray crystallography (coding strand in green and non-coding strand in red). The transcription start site is identified as a white base pair and the sites of TFIIA crosslinking to the AdMLP are shown as blue nucleotides. The portion of the upper surface of TBP2 implicated in TFIIA binding is also coloured blue and includes positively charged side chains. *a*, Viewed parallel to the axis of rough intramolecular 2-fold symmetry. The horizontal displacement of the two helix axes caused by the protein-DNA interaction is obvious. *b*, Viewed at 90° with respect to that of *a*. The overall DNA bend induced by TBP2 binding is 80°.



approximate 2-fold symmetry and do not necessarily explain directional DNA binding by TBP. Between the second and tenth base pairs of the co-crystallization oligonucleotide the sequence reads CTATAAAAG in one orientation and CTTTATAG in the other, suggesting that protein-DNA contacts at positions -30, -28 and -26 (indicated with bold lettering) of the AdMLP determined DNA-binding polarity. Changing A→T→A at positions -30, -28 and -26 reduces yeast TBP activity *in vitro* to 60%, 25% and 10%, respectively³³, implying that Phe 148, Phe 165, Leu 163, Thr 173, Val 19, Thr 82 and Val 80 specify the direction of TBP binding (Fig. 4c). Three of these side chains make van der Waals interactions with each of the adenine bases in question (Leu 163-A(-30)=3.6 Å, Val 119-A(-28)=3.7 Å, Val 80-A(-26)=3.5 Å), fixing the direction of TATA-box binding by distinguishing A from T at each of these non-symmetrical positions.

The importance of sequence-specific DNA deformation and protein conformational changes during protein/nucleic acid recognition is widely acknowledged, albeit poorly understood (reviewed in ref. 36). The most extreme examples include CAP³⁷ and E2 (ref. 38), which both wrap B-form DNA around the protein's convex surface using major groove and phosphate-ribose DNA contacts. In contrast, TBP-TATA box complex formation involves remodelling of DNA to the concave surface of the protein and partial unwinding of the double helix, permitting side-chain/base interactions in the widened minor groove.

The transitions from B-form to partially unwound DNA and back occur abruptly at two sharp kinks towards the major groove, which are, respectively, stabilized by Phe 148 and Phe 165 inserting into the T·A:A·T step and Phe 57 and Phe 74 inserting within the A·T:G·C step. Site-directed mutagenesis of three of these four critical residues in yeast TBP abolishes DNA binding in gel shift assays (Phe 57, Phe 74→Leu, Phe 74→Leu or Tyr, Phe 165→Leu or Tyr)¹³. Kink formation is probably not sequence specific because a variety of TATA-element mutations at either kink site are tolerated in *in vitro* transcription initiation assays with yeast TBP³³. Between the two kinks, base-pair steps within the sequence ATAAAA show significant positive roll, creating a smooth bend towards the major groove (Fig. 2b). Various single base-pair mutations, including some G→C and C→G substitutions, are also tolerated at these positions^{14,33}, where they do not appear to introduce unfavourable side-chain/base minor groove contacts (see above). TBP-induced conformational changes in the TATA element must, therefore, result from both sequence-dependent DNA deformability and side-chain/base minor groove contacts.

We do not yet have a detailed thermodynamic picture of TBP-DNA binding, but there is biochemical evidence that both TBP and the TATA element undergo conformational changes on binding^{17,39,40}. Kinetic data suggest that TBP binding to the TATA box proceeds by two steps, involving a low-affinity protein-DNA complex ($K_d = 10^{-7}$ M) followed by formation of the

productive, nanomolar-affinity complex⁴⁰. Our co-crystal structure confirms that both TBP2 and the oligonucleotide undergo conformational changes on binding and emphasizes the need for further X-ray crystallographic and physical-chemical studies of this and other TBP-DNA complexes. Specific binding of TBP to DNA may simply result from induced fit of both the protein and nucleic acid, provided no unfavourable steric overlaps develop in the extensive TBP-DNA interface. This DNA-binding mode could explain TBP-mediated pol II transcription from TATA-deficient promoters (reviewed in ref. 41).

Promoter interactions and transcription

TATA element deformation by binding of TBP2 has implications both for preinitiation complex assembly/stabilization and for transcription activation. A bend in the core promoter centred on the TATA box would permit the general transcription initiation factors and pol II to form a closer association than they would on linear DNA. Figure 5 shows two orthogonal views of a model of TBP2 bound to the AdMLP in which linear B-form DNA has been added to each end of the co-crystallization oligonucleotide. The model displayed in Fig. 5 also depicts regions of both TBP^{42,43} and the AdMLP (J. Greenblatt, personal communication) implicated in binding TFIIA, which stabilizes TBP-DNA interactions^{10,42}. DNase I footprinting studies documented TFIIB protection of positions -10 to +10 in a TBP-TFIIA-TFIIB-AdMLP complex. Subsequent addition of pol II extended the footprint to positions +20 and -47, on either side of TBP¹⁰. Crosslinking studies have also localized RAP30, a subunit of yeast TFIIF that recruits pol II to the preinitiation complex^{10,44}, to position -19 of the AdMLP (J. Greenblatt,

personal communication). The unusual DNA conformation induced on TBP2 binding could also explain, at least in part, the ability of upstream activator proteins to interact with TBP (reviewed in ref. 45).

Our structure provides a ready explanation for competition between nucleosome formation and pol II-mediated transcription initiation. Assembly of the AdMLP into nucleosomes represses basal transcription (reviewed in ref. 46), whereas a preformed preinitiation complex remains transcriptionally active after nucleosome assembly and recombinant yeast TBP alone prevents nucleosome-mediated repression of transcription⁴⁷. Earlier predictions that short A+T-rich sequences favour bending towards the minor groove and positioning of the minor groove towards the histone proteins in chromatin^{48,49} suggest that direct competition between TBP and the histone core for the minor groove of the TATA element is possible. Given that TBP bends the TATA element in a direction opposite to that preferred in the nucleosome, an existing preinitiation complex would affect local chromatin structure⁴⁶. TBP may also compete with anti-tumour drugs such as netropsin and distamycin A, which bind in the minor groove to A+T-rich sequences^{50,51}.

In conclusion, the three-dimensional structure of this highly unusual protein/nucleic acid complex explains many of the DNA-binding properties of TBP. Our work also provides a starting point for further crystallographic, biochemical and genetic studies of TBP and its role in pol II-mediated transcription initiation. In particular, these results should aid directed, systematic analyses of the mechanisms by which TBP recognizes TATA boxes through DNA deformation and minor groove contacts and modulates class II nuclear gene promoter structure. □

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Detection of pulsed X-rays from the binary millisecond pulsar J0437 – 4715

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THE X-ray properties of pulsars (highly magnetized rotating neutron stars) depend mainly on the age of the star¹. For young ($\leq 10^4$ yr) pulsars, virtually all of the X-ray emission is pulsed, appearing as sharp bursts with a power-law spectrum; these emissions are thought to arise from the acceleration of electrons and positrons in the pulsar's magnetosphere^{2–5}. Older pulsars, on the other hand, exhibit broad X-ray pulses, a lower pulsed fraction and black-body spectra; this is ascribed to thermal emission from the surface of the cooling neutron star, the modulation of the X-rays reflecting temperature inhomogeneities induced by the strong magnetic fields^{6,7}. No thermal emission is expected for neutron stars older than $\sim 10^6$ yr (refs 8–10) in the absence of surface heating processes^{11–15}. Here we report the discovery of pulsed X-ray emission from a $\sim 2 \times 10^9$ -yr-old neutron star: the binary millisecond pulsar J0437 – 4715. The spectral properties of the pulsed emission suggest a thermal origin, requiring substantial heating of the neutron-star surface either by internal friction^{11–13} or by energetic particles streaming onto the polar cap from the pulsar's magnetosphere^{14,15}.

The pulsar J0437 – 4715 was recently discovered during a radio survey of the southern sky for millisecond pulsars¹⁶. At a distance of ~ 140 pc it is the closest known millisecond pulsar; with a rotational period of 5.75 ms and a 5.74-day circular orbit with a low-mass companion of ~ 0.2 solar masses ($M_\odot$)^{16–19}. The system is not eclipsing¹⁶ and no optical brightness variation as a function of orbital phase could be detected from the companion^{18,19}.

PSRJ0437 – 4715 was observed with the position sensitive proportional counter (PSPC) on the Rosat satellite between 30 July and 2 Aug 1990 as part of the Rosat all-sky survey¹⁷, and between 20 and 21 September 1992 as a serendipitous target in a pointed observation of the active galactic nucleus (AGN) RX J0437 – 4711. During the latter observation it was ~ 4 arcmin off-axis with an effective exposure time of 5,846 s. Here we concentrate on this latter observation which consists of three exposures at orbital phases 0.84, 0.01 and 0.02 with respect to the ascending node.

A spatial fit to the data was obtained from a spline fit to the background level in combination with a maximum-likelihood analysis of the source counts. The dead time- and vignetting-corrected net count rate deduced from this is 0.204 ± 0.006 counts s^{-1} in the (0.1–2.4)-keV range. No significant orbital modulation could be detected. The source location is estimated to be only 4.2 arcsec from the pulsar's radio position¹⁶, consistent with the PSPC positional errors. There is no evidence of extended emission. The given count rate improves the all-sky survey results¹⁷ where the source flux was underestimated because of poor counting statistics and contamination from the nearby AGN source.

To test the detected X-ray flux for a modulation at the pulsar's radio frequency, a photon-arrival time analysis was applied to 1,200 photons, selected from a 70 arcsec aperture centred on the pulsar's X-ray position. For the reduction of the spacecraft coordinates and event times to the Solar System barycentre (SSB) and the barycentric dynamical timescale (TDB), we performed the standard barycentring procedures^{20–22} with respect to the pulsar's binary nature, using the JPL DE200 Earth ephemeris²³ in combination with the pulsar's radio position. Given the extreme timing stability of millisecond pulsars in com-

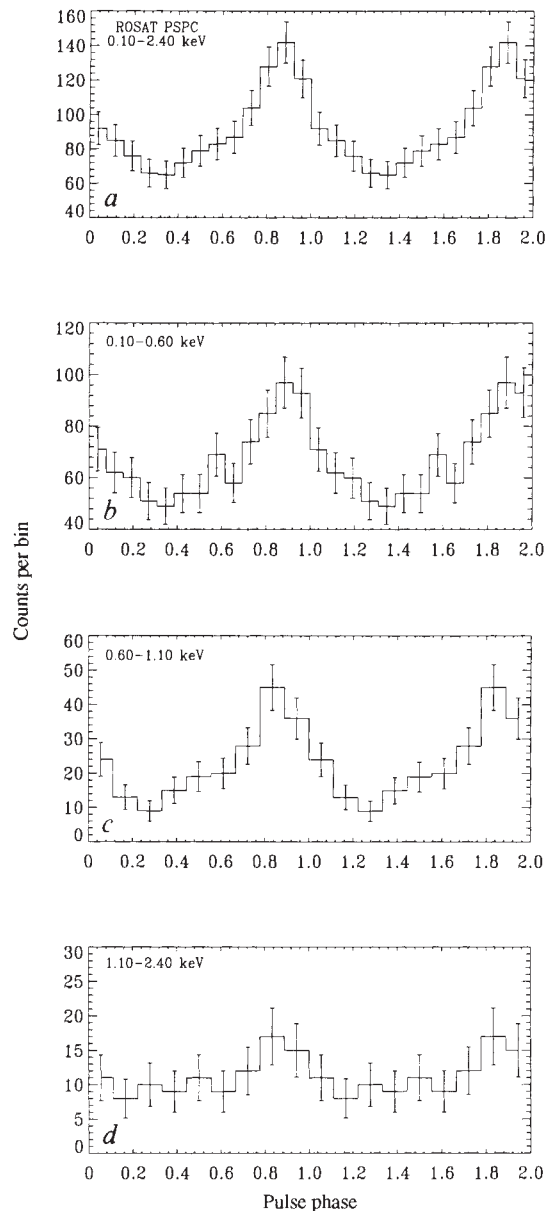


FIG. 1 The X-ray light curves of PSRJ0437 – 4715 determined for different energy ranges: 0.10–2.40 keV (a), 0.10–0.60 keV (b), 0.60–1.10 keV (c), and 1.10–2.40 keV (d). The data have been divided into 13 phase bins for a and b, and nine phase bins for c and d. Two phase cycles are shown for clarity and 1σ errors are indicated. Phase 0 corresponds to $t_{\text{ref}} = 2448886.9802187979$ TDB. The pulse shape is characterized by a single broad peak and an energy-dependent pulsed fraction of $(30 \pm 3)\%$ for b, $(53 \pm 6)\%$ for c and $(27 \pm 9)\%$ for d. The averaged pulsed fraction within (0.1–2.4) keV (a) was found to be $(33 \pm 3)\%$.

TABLE 1 Spectral models

Model	Column density (atoms cm^{-2})	Parameters	$L_x(d=140 \text{ pc})$ (0.1–2.4) keV
Power law	$(1.4 \pm 0.5) \times 10^{20}$	$\Gamma = -2.6 \pm 0.2$	$6.0 \times 10^{30} \text{ erg s}^{-1}$
Exponential	$(0.5 \pm 0.3) \times 10^{20}$	$T = (5.3 \pm 0.7) \times 10^6 \text{ K}$	$3.3 \times 10^{30} \text{ erg s}^{-1}$
Thermal Bremsstrahlung	$(0.6 \pm 0.3) \times 10^{20}$	$T = (6.6 \pm 1.0) \times 10^6 \text{ K}$	$3.6 \times 10^{30} \text{ erg s}^{-1}$
Composite model			
Power law	$(1.0 \pm 0.5) \times 10^{20}$	$\Gamma = -2.85 \pm 0.2$	$3.7 \times 10^{30} \text{ erg s}^{-1}$
Black body		$T \sim 1.7 \times 10^6 \text{ K}$	$1.3 \times 10^{30} \text{ erg s}^{-1}$

For the black-body model, L is the bolometric luminosity.

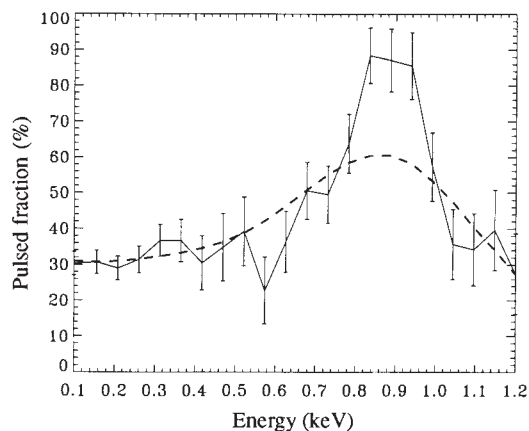


FIG. 2 The fraction of pulsed photons, deduced from a sine wave fit to the light curve in an energy window $E_c \pm 0.15$ keV, as a function of the central window energy E_c (solid line). 1σ -errors are indicated. Horizontal bars to indicate the energy selection range are suppressed for clarity. To overcome the effects of energy oversampling and to match the relevant PSPC energy resolution ($\sim 43\%$ full-width half maximum (FWHM) at 1 keV), the curve was also convolved with the detector response function. The folded curve (dashed line) is a more realistic description of the energy dependent pulsed fraction of PSRJ0437-4715, which peaks at $E_c \sim 0.9$ keV.

binning with the precision of the known radio frequency and its first time derivative, the radio ephemeris¹⁶ could be extrapolated to the epoch of the Rosat observation dates. Consequently, each X-ray photon arrival time could be directly related to the pulsar phase ϕ , using the relationship $\phi_i = \text{fractional part of } (f\Delta t_i + \frac{1}{2}\dot{f}\Delta t_i^2)$ with $i = 1$ to N , where N is the number of photons, f is the extrapolated pulsar frequency 173.687949350 Hz at $t_{\text{ref}} = 2448886.9802187979$ JD, $\dot{f}$ its first time derivative $-3.8011054728 \times 10^{-15}$ Hz s^{-1} and $\Delta t_i = t_i - t_{\text{ref}}$. The resultant light curve for the energy range (0.1–2.4) keV is shown in Fig. 1a. The data have been divided into 13 phase bins, and two phase cycles are shown for clarity. The pulse shape is characterized by a single broad peak and an averaged pulsed fraction of $(33 \pm 3)\%$.

Using the Z_n^2 -test (ref. 24) with $n = 1$ to 10 harmonics, we found that the statistical significance for pulsations in the total energy range (0.1–2.4) keV is greatest for $n = 2$ with $Z_2^2 = 75.9$, corresponding to the low harmonic content of the pulse shape. As the test statistic Z_n^2 is distributed (in the absence of a signal)

as χ^2 with $2n$ degrees of freedom, the formal probability of finding the X-ray flux modulated by chance with the radio frequency is $\sim 10^{-12}$, including the trials involved in optimizing the longitude of periastron ω to 5.03° . This result establishes PSRJ0437-4715 as the first millisecond X-ray pulsar, one of the rare pulsed species that have been detected in both radio and X-ray wavebands^{25,26}. (Although the first millisecond pulsar detected in X-rays was the 'black widow' pulsar PSR1957+20 (refs 27, 28), the low X-ray flux of the observations unfortunately precluded any detailed source analysis and periodicity testing, so that the question of whether the X-rays originate in this pulsar has not yet been solved.)

To investigate a possible spectral variation of the pulse phase and pulsed fraction, the timing analysis was repeated for a 0.3-keV energy window, varying between 0.1 and 1.2 keV. Above 1.2 keV, the analysis was limited by the low photon flux so that the pulsed fraction could only be estimated in the range (1.2–2.4) keV (compare with Fig. 1d). The results of the analysis are summarized in Fig. 2, where it can be seen that the pulsed fraction varies with energy and peaks in the energy range (0.6–1.1) keV. This is a remarkable result and means that the pulsed component dominates the spectrum in the narrow band (0.6–1.1) keV. A similar result was recently reported for the 197-ms pulsar PSR1055-52 (ref. 29). However, an energy-dependent phase shift (as found for PSR1055-52 and the X- and gamma-ray pulsar Geminga³⁰) could not be detected (Fig. 1b–d).

To investigate the spectral characteristics of PSRJ0437-4715, various spectral models were fitted to the measured energy distribution (Table 1). The total spectrum was found to be represented best by a power law with photon index $\Gamma = -2.6 \pm 0.2$ and column density $N_H = (1.4 \pm 0.5) \times 10^{20}$ atoms cm^{-2} (Fig. 3a). The value expected from the radio dispersion measure ($DM = 2.6534$ pc cm^{-3}) is about $(0.8-0.9) \times 10^{20}$ atoms cm^{-2} , assuming ten hydrogen atoms for each free electron. A single thermal spectrum does not fit the distribution and leaves a residual hard excess above 0.4 keV. The peak in the energy-dependent pulsed fraction supports the idea, however, that a two-component spectral model may be more appropriate. Our first choice was a power law, representing magnetospheric or nebular emission, superimposed on a black-body component caused by thermal emission from the neutron-star surface (Table 1). This model fits the data very well (Fig. 3b) and is able to explain the energy-dependent pulsed fraction. The best-fit column density is $N_H = (1.0 \pm 0.5) \times 10^{20}$ atoms cm^{-2} , in agreement with the value expected from the dispersion measure. The temperature of $\sim 1.7 \times 10^6$ K and the luminosity $L_{\text{bol}} \sim 1.3 \times 10^{30}$ erg s^{-1} correspond to a radiating surface area of 0.05 km², implying the exist-

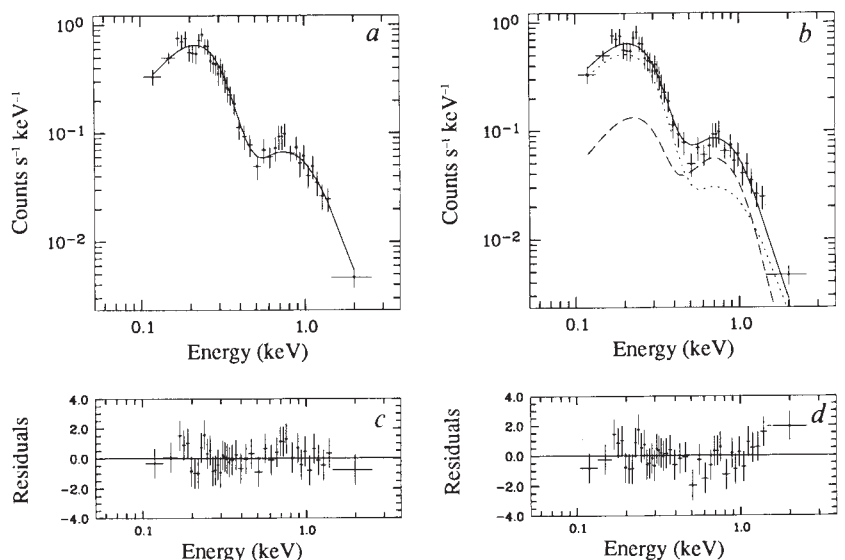


FIG. 3 Spectral fits of a power law (a) and a composite model (b). The latter consists of a power law (dotted line) plus a thermal component (dashed line). The data were binned into 42 energy channels with respect to a signal to noise ratio of $(4-6)\sigma$. However, because this is still below the PSPC energy resolution which is $\sim 43\%$ (FWHM at 1 keV) the different data points may not be all independent from each other and are affected by some energy oversampling. The feature at 0.2 keV may not be significant for that reason. The residuals (c, d) are in units of 1σ .

ence of a relatively small hot-spot on the neutron-star surface.

In summary, the data suggest that a significant fraction of the X-rays are due to pulsed thermal emission which probably comes from a polar hot-spot. Compared with the other pulsars from which pulsed thermal emission has been detected (Vela, PSR0656 + 14, PSR1055 - 52 and Geminga)²⁵, PSRJ0437 - 4715 has a considerably larger characteristic age ($P/2\dot{P} \sim 2 \times 10^9$ yr). This strongly supports the idea that after neutrino and photon cooling, neutron stars are kept hot either by internal frictional heating^{11, 13} or by bombardment of the polar cap by energetic particles accelerated in the magnetosphere^{14, 15}. The possibility that the heating occurred during a past accretion phase seems unlikely because the cooling time of a neutron star is much shorter than the characteristic age of this pulsar. The ratio of pulsed X-ray luminosity to the spin-down power is $\sim 10^{-4}$, consistent with the models of reheating and polar cap activity. $\square$

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Synthesis, structure and activity of artificial, rationally designed catalytic polypeptides

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BIOLOGICAL macromolecules with catalytic activity can be created artificially using two approaches. The first exploits a system that selects a few catalytically active biomolecules from a large pool of randomly generated (and largely inactive) molecules. Catalytic antibodies¹ and many catalytic RNA molecules² are obtained in this way. The second involves rational design of a biomolecule that folds in solution to present to the substrate an array of catalytic functional groups³⁻⁸. Here we report the synthesis of rationally designed polypeptides that catalyse the decarboxylation of oxaloacetate via an imine intermediate. We determine the secondary structures of the polypeptides by two-dimensional NMR spectroscopy. We are able to trap and identify intermediates in the catalytic cycle, and to explore the kinetics in detail. The formation of the imine by our artificial oxaloacetate decarboxylases is three to four orders of magnitude faster than can be achieved with simple amine catalysts: this performance rivals that of typical catalytic antibodies.

Decarboxylation of oxaloacetate is the final reaction in the Rozzell process for the industrial synthesis of phenylalanine⁹. This process achieves decarboxylation by means of a natural enzyme (an oxaloacetate decarboxylase) that requires a metal ion as a cofactor¹⁰. Because industrial processes may be incon-

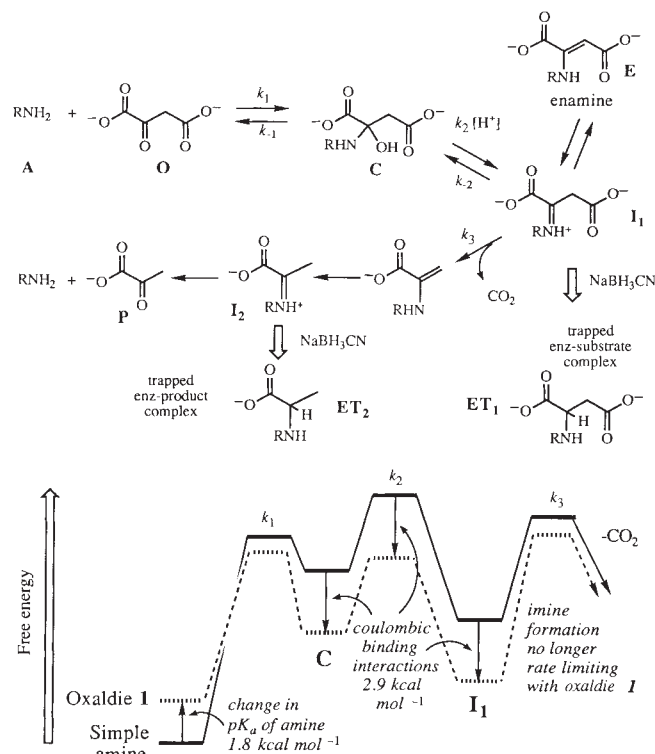


FIG. 1 Kinetic scheme and an approximate free-energy profile describing the decarboxylation of oxaloacetate catalysed by amines (R defined in Table 1). Open arrows indicate reactions used to trap intermediates in the catalytic cycle. The free-energy profile is for butyl amine (solid line) and oxaldie 1 (broken line), and shows the points where the designed peptide catalyst alters the free-energy profile to achieve rate acceleration. The relative free energies of bimolecular states are calculated using a 1 M standard state, corrected for amount of free amine. A, Amine; O, oxaloacetate; C, carbinolamine; P, pyruvate; I₁, imine intermediate between amine and oxaloacetate; I₂, imine intermediate between amine and pyruvate; ET₁, trapped imine with oxaloacetate; ET₂, trapped imine with pyruvate.

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veniened by cofactors, a metal-free oxaloacetate decarboxylase that operates by a different mechanism would be desirable.

Alternative mechanisms are conceivable: for example, oxaloacetate can be decarboxylated by amines that react to form an imine, which then loses carbon dioxide (Fig. 1)^{11–14}. But no natural oxaloacetate decarboxylase is known to use the imine mechanism, presumably because the metal-dependent mechanism is evolutionarily optimal for this particular substrate^{15,16}. Design would seem to be the only way to obtain a metal-free oxaloacetate decarboxylase that operates by an imine mechanism.

To design a new peptide to catalyse the decarboxylation of oxaloacetate via an imine (we call the peptide an 'oxaldie'), the mechanisms of amine-catalysed decarboxylation in general must be understood. Six features relevant to the design emerged by combining published information^{11–14,17} with details of the kinetics of the decarboxylation of oxaloacetate catalysed by five model amines¹⁸. (1) Decarboxylation can proceed via an imine intermediate (Fig. 1)^{11–14}. The new peptide catalyst should therefore carry an active-site amino group capable of forming an imine with oxaloacetate. When constrained to use only those amino acids encoded naturally by messenger RNA, this must be either the amino-terminal amine of the polypeptide itself (strategy (a)) or the ϵ -amino group of a constituent lysine (strategy (b)). (2) Imine formation is largely rate-determining for decarboxylation above pH 4 (refs 18, 41). Speeding imine formation therefore becomes the focus of the design. (3) The rate-determining step in imine formation between pH 4 and 7 is the specific acid-catalysed elimination of water from carbinolamine C ($k_2[H^+]$ in Fig. 1)^{17–20}. This implies that general acids need not be built into the active site to speed imine formation. (4) Carbinolamine C is the high-energy intermediate along the reaction coordinate. Thus the rate of imine formation can be accelerated by introducing stabilizing binding interactions between the peptide and carbinolamine C (and therefore, according to the Hammond postulate, the transition state that follows)^{18,21,41}. (5) The catalytically most effective amine has a pK_a equal to the ambient pH. For each log unit decrease in its pK_a , the intrinsic catalytic power of an imine decreases by half a log unit (a Bronsted β of 0.5)^{18,22}, but the concentration of free amine (the reactive species) increases by a full log unit. This implies that the catalytic effectiveness increases by half a log unit until the pK_a of the amine reaches the ambient pH. (6) The partition ratio of the imine intermediate towards product and back to substrate (k_3/k_2 in Fig. 1) is independent of both the pH and the pK_a of amine¹⁸. Thus, perturbing the pK_a of the amine will not influence the partitioning unfavourably for catalysis.

The scaffolding for holding a reactive amine is an amphiphilic α -helix^{23–28} capable of building helix bundles in aqueous media (Fig. 2)²⁹. Positively charged residues were attached to the helices to bind the dianionic carbinolamine C (Fig. 1) by coulombic interactions. Two strategies were explored to lower the pK_a of the amino group. In strategy (a) (yielding the peptide oxaldie 1), the terminal amino group of the polypeptide chain is left free to serve as the reactive amine. Its pK_a is depressed by interactions with the 'helix dipole'^{30–32} when a helix is formed. In strategy (b) (yielding oxaldie 2), the amino-terminal amine is blocked; the reactive amino group must therefore come from a lysine side chain whose pK_a is depressed by coulombic interaction with neighbouring protonated lysine side chains when the helix is formed.

The peptides were synthesized by solid-phase methods (Fig. 2). Circular dichroism spectra indicate that oxaldies 1 and 2 are, respectively, 18 and 33% α -helical in aqueous buffer at infinite dilution; the different helical content relates to their relative stabilities as predicted from the helix dipole, which interacts unfavourably with the free amino-terminal amine in oxaldie 1 (refs 30–32). Helicity is a strong function of peptide concentration, however, suggesting that the α -helices are stabilized by

aggregation^{24–29}. The size of the aggregates formed was estimated by concentration studies^{24–28} and size-exclusion chromatography. Oxaldie 1 seems to aggregate to form a four-helix bundle first, whereas oxaldie 2 forms larger aggregates.

Secondary structures of both peptides at 8 mM were established by two-dimensional NMR spectroscopy. Helical segments were identified from nuclear Overhauser effects (NOEs) of the type d_{NN} , $d_{\alpha N}(i, i+3)$, and $d_{\alpha N}(i, i+4)$ (ref. 33). Such cross-peaks are observed for all but the first four residues of oxaldie 1 in 20% aqueous trifluoroethanol, a structure-forming solvent³⁴. For oxaldie 2, NOEs indicative of α -helical secondary structure were observed for the entire length of the peptide without trifluoroethanol, again indicating greater stability in the terminally acetylated helix. Titration identified a single amino group in oxaldie 1 with a pK_a of 7.2, assigned as the terminal amine by trapping with acetic anhydride^{35,36}. This pK_a is depressed 0.6 units by helix formation (relative to Leu-Ala-NH₂)¹⁸. The lowest pK_a in oxaldie 2 is 8.9 (1.6 units below that of a typical lysine).

Both oxaldies 1 and 2 catalyse the decarboxylation of oxaloacetate (Table 1) with Michaelis–Menten saturation kinetics ($k_{cat} = 0.4 \text{ min}^{-1}$ and 0.5 min^{-1} ; $K_m = 14 \text{ mM}$ and 48 mM , respectively). By coincidence, the first k_{cat} is the same as that reported for the first catalytic antibody³⁷. Imines I₁ and I₂ (Fig. 1) with oxaldie 1, proposed as intermediates in the catalytic cycle, were trapped with NaBH₄CN. Rates were not increased by external general acids or bases. Monoanionic β -ketoacids (for example, acetoacetate) were not substrates, and acetone dicarboxylate, acetate and malate were not inhibitors. Pyruvate, the product of the decarboxylation, weakly inhibited oxaldie 1, ($K_i \approx 70 \text{ mM}$).

When both the amount of trifluoroethanol³⁴ and the concentration of the peptides are varied, catalytic activity correlates with the fraction of helix formed. This suggests that the catalytically active forms of the oxaldies are the helices. To confirm this, the helical structure of oxaldie 2 was disrupted by mutating leucine residues at positions 4, 8 and 11 to Gly, Pro and Gly, respectively. Unlike oxaldie 1 and 2, the mutant peptide was found by circular dichroism to adopt random-coil conformation at all concentrations; its catalytic activity is decreased by an

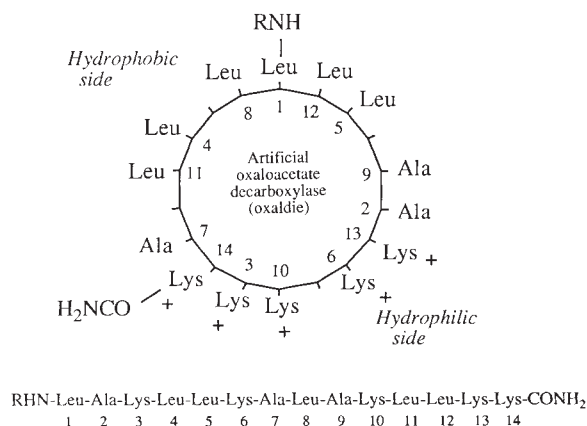


FIG. 2 Sequences of two designed artificial oxaloacetate decarboxylases (oxaldie 1 and oxaldie 2; R for oxaldie 1 is hydrogen, and for oxaldie 2 is an acetyl group together with a representation of these structures on a helical wheel to illustrate the amphiphilic nature of the helices that they build. Five amino groups from the side chains of lysine protrude on one face of the helix. In oxaldie 1, the amino-terminal amine is the principal active site. In oxaldie 2, the ϵ -lysine amino groups provide the active site. Both peptides were synthesized using standard *t*-Boc chemistry⁴⁰ on polystyrene/1% divinylbenzene resin with the *p*-methylbenzhydrylamine linker. Deprotection of the peptide and release from the support were achieved by treatment with trifluoromethylsulphonic acid in trifluoroacetic acid in the presence of ethanedithiol and thioanisole. Peptides were purified by reversed-phase C₁₈ HPLC.

TABLE 1 Comparison of kinetic parameters for decarboxylation of oxaloacetate with oxaldie and some model amines*

Catalyst	pK _a (lowest)	Trifluoro- ethanol (v/v)	k _{cat} /K _m or k _{obs} (s ⁻¹ M ⁻¹)	log relative k _{cat} /K _m (or k _{obs})	k _{cat} × 10 ³ (s ⁻¹)	K _m (mM)	k _{im} (s ⁻¹ M ⁻¹)	log relative k _{im}
Oxaldie 1	7.2	5%	0.63	2.8	5.5	8.7	2.6	3.4
Oxaldie 1	7.2	—	0.47	2.6	6.7	14.2	—	—
Oxaldie 2	8.9	5%	0.21	2.3	6.8	33	—	—
Oxaldie 2	8.9	—	0.15	2.1	7.5	48	—	—
Mutant	9.9	5%	0.028	1.4	—	—	—	—
Mutant	9.9	—	0.022	1.3	—	—	—	—
PheOEt	7.2	5%	0.013	1.1	—	—	—	—
PheOEt	7.2	—	0.012	1.0	—	—	0.024 [†]	1.3
Butylamine [‡]	10.6	—	0.0011	0.0	—	—	0.0011	0.0
Spontaneous	—	—	—	—	0.013	—	—	—

All parameters determined at pH 7, 25 °C, ionic strength = 0.15 (NaCl) and [peptide] = 0.2 mM. Rates were measured by following either the loss of absorbance at 285 nm arising from the enol of oxaloacetate (oxaldie 1, mutant, and simple amines) or the rate of formation of pyruvate using lactate dehydrogenase (oxaldie 1, oxaldie 2) in aliquots treated first with malate dehydrogenase (necessary because of the residual catalytic activity of lactate dehydrogenase with malate).

* Oxaldie 1 is H₂NLeuAlaLysLeuLeuLysAlaLeuAlaLysLeuLeuLysLysCONH₂; oxaldie 2 is AcNHLeuAlaLysLeuLeuLysAlaLeuAlaLysLeuLeuLysLysCONH₂; Mutant is CH₃CONHLeuAlaLysGlyLeuLysAlaProAlaLysGlyLeuLysLysCONH₂; PheOEt is phenylalanine ethyl ester; k_{im} is rate of imine formation. Michaelis–Menten kinetics were observed up to an oxaloacetate concentration of 30 mM. At higher concentrations, oxaldie 1 catalyses a Mannich reaction between two molecules of oxaloacetate. Rate data for oxaldie 1 are corrected for catalytic activity arising from the lysine side chains.

[†] Interpolated for an amine with a pK_a of 7.2, with correction for steric hindrance.

[‡] Extrapolated from ionic strength of 1.

order of magnitude, and it no longer follows Michaelis–Menten saturation kinetics.

Upon mixing oxaldie 1 with oxaloacetate, a transient species was observed by ultraviolet spectroscopy (at 290 nm) in a pre-steady-state 'burst'. This was assigned to enzyme-bound enamine (*E* in Fig. 1)^{38,39}. The formation of enamine is first-order in oxaloacetate and is negligibly catalysed by buffers, suggesting that imine formation limits the rate of enamine formation^{13,39}. Analysis of the burst suggests that the rate of imine formation in oxaldie 1 is 3–4 orders of magnitude higher than for a simple amine with a pK_a of 10.6 (Table 1).

Natural enzymes catalyse reactions by using the conformational energy of their polypeptide fold to force functional groups in the polypeptide into environments where their reactivity is enhanced, and by exploiting binding between the enzyme and non-reacting portions of the substrates to lower the energy of a transition state relative to a state with free enzyme and substrate²¹. The oxaldies seem to exploit both mechanisms to achieve catalysis. First, placing the reacting amine near the end of a helix (in oxaldie 1) or adjacent to positively charged side chains (in oxaldie 2) lowers its pK_a and increases its reactivity. This contributes more to catalysis in oxaldie 2 (ΔpK_a = 1.6) than in oxaldie 1 (ΔpK_a = 0.6) (Table 1).

But, this alone does not account for catalysis, as imine I₁ is formed with oxaldie 1 100 times faster than expected for an amine with a pK_a of 7.2 (Table 1). The additional catalytic power is probably due to coulombic binding between the cationic peptide and the anionic transition state following carbinolamine C. The dissociation constant of substrate (in all forms) from oxaldie 1, estimated from saturation kinetics of the burst, offers an estimate of the magnitude of these interactions. The dissociation constant for oxaldie 1 ([oxaloacetate] [oxaldie 1] / [complexes]) is ~6 mM. This is four times lower than the K_s dissociation constant estimated (20 mM) for the complex between oxaloacetate and ethylenediamine¹³ (where one positive charge on the amine contributes to binding), and far lower than analogous dissociation constants for simple amines (~1 M)¹² in which no positive charges stabilize the intermediate.

Together these factors yield a rate of formation of imine that is 3–4 orders of magnitude larger with oxaldie 1 than with simple amines. This is within the range of rate enhancements achieved by selection strategies that yield catalytic antibodies (10³–10⁶) (ref. 1), and is not insignificant when compared with the 10⁸-fold rate enhancement in the natural (metal-dependent) oxaloacetate decarboxylases used in the Rozzell process⁹. Remarkably, an explicitly designed binding pocket is not necessary to achieve

these rates. However, an understanding of the kinetics and mechanism of the process is helpful both for guiding design and for understanding what has transpired as a result of the design. From our analysis, imine formation seems not to be rate-determining in the catalytic cycle in oxaldie 1, so efforts to enhance the catalytic power of these peptides must focus on other steps in the reaction sequence. □

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Stratospheric transport from the tropics to middle latitudes by planetary-wave mixing

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TRANSPORT of air from the troposphere to the stratosphere takes place mainly in the tropics¹. By studying satellite records of the dispersal of volcanic aerosols from tropical eruptions, Trepte and Hitchman² concluded that there is a barrier inhibiting the transport of stratospheric air from the tropics to middle latitude, raising the question of how stratospheric material that has been transported from the troposphere is subsequently conveyed to higher latitudes. Here we present global maps of nitrous oxide and water mixing ratios obtained by the Upper Atmosphere Research Satellite. We see strong latitudinal gradients in these trace species, confirming the existence of a barrier to transport. But superimposed on this background structure we also see planetary-scale 'tongues' of tropical stratospheric air extending out into middle latitudes, and time sequences show irreversible mixing from the tropics into middle latitudes. Such episodes could be responsible for transporting significant quantities of stratospheric air across the tropical barrier.

Two long-lived stratospheric trace constituents are analysed here, namely nitrous oxide (N_2O) and water vapour (H_2O). Data on N_2O are obtained by the Cryogenic Limb Array Etalon Sounder (CLAES) on the Upper Atmosphere Research Satellite (UARS)³, and data on H_2O are obtained from a separate instrument on UARS, the Microwave Limb Sounder (MLS)⁴. Identification of the wave structures in these independent data increases confidence in their reality, and provides a means of validating them. The satellite yaws at intervals of ~ 1 month, providing coverage from 30° latitude in one hemisphere to 80° in the other for successive periods. Orbiting-satellite data are mapped on standard pressure surfaces using a Kalman Filter technique⁵, which results in daily maps of the constituent fields. The data are mapped with a $\sim 4^\circ$ latitude resolution, and a longitudinal truncation at zonal wavenumber 6 ($\sim 30^\circ$ longitudinal resolution); the vertical resolution is ~ 2.5 km. Because of higher noise levels in the CLAES data, they are smoothed slightly in latitude and height before plotting.

The longitudinally averaged structure of the N_2O distribution during 1–20 September 1992 is shown in Fig. 1 (data north of 30° N were taken from the previous north-looking yaw interval). The source region for N_2O is near the Earth's surface. Nitrous oxide is well mixed in the troposphere (the lowest 15 km of the atmosphere), and decays with height in the stratosphere due to photochemical destruction above 40 km. The photochemical lifetime of N_2O below 40 km is >100 d (several years below 30 km), so that the distribution in the stratosphere is determined mainly by the circulation. As shown schematically in Fig. 1, rising motion in the tropics is associated with a maximum in N_2O over $\sim 10^\circ$ S to 30° N, and sinking motions over high latitudes of both hemispheres result in relatively low N_2O values. This downward displacement is particularly large over the South Pole at this time of year, so that the polar vortex is reflected in strong latitudinal N_2O gradients; rather more pronounced gradients across the Southern Hemisphere polar vortex are observed in UARS methane (CH_4) observations⁶. Horizontal transport by

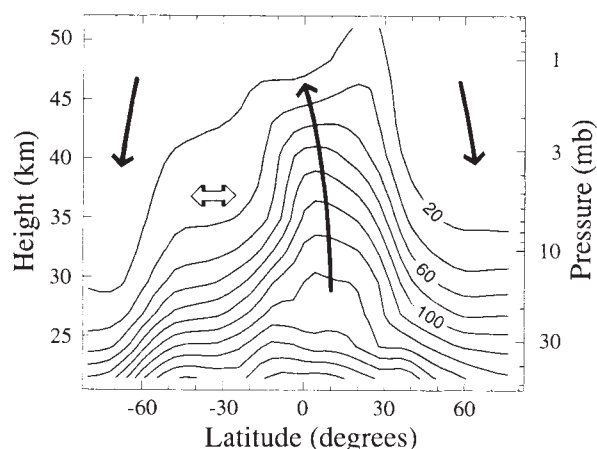


FIG. 1 Longitudinally averaged structure of nitrous oxide (N_2O) mixing ratio (in parts per billion (10^9 by volume (p.p.b.v.)) during 1–20 September 1992 measured by the CLAES instrument on UARS. Heavy solid lines denote the mean stratospheric circulation in the latitude–height plane, and the horizontal arrows denote the location of quasi-horizontal mixing by planetary waves.

planetary waves in the winter stratosphere results in a well mixed region between midlatitudes and subtropics, known as the 'surf zone'^{7,9}; this is seen in Fig. 1 as the region of relatively flat N_2O gradients over 20 – 50° S. This overall structure of the N_2O distribution is in good agreement with previous satellite observations¹⁰, and with idealized models of the mean stratospheric circulation that include the effects of planetary-wave transport¹¹.

Figure 2 shows a sequence of horizontal maps of the global N_2O distribution over 30° N– 80° S on the 1,100 K isentropic surface (altitude ~ 38 km). Superimposed on the background structure of high values in the tropics is a tongue of high- N_2O air extending from the tropics deep into midlatitudes, with a distinctive NW–SE phase tilt. Similar tongue-like features have been found in satellite measurements of stratospheric ozone⁹ and aerosol¹², and have also been noted in three-dimensional simulations of stratospheric tracers^{12,13,23}. This characteristic tongue-like structure is observed from late August throughout the middle of September, when it remains fixed geographically but modulates in intensity. The time period in Fig. 2 is near the amplitude maximum, and perturbations associated with the tongue are discernible between the equator and 50° S. Similar wave patterns are observed at all vertical levels over ~ 28 – 42 km, which is the altitude range over which the strong subtropical N_2O gradients are found (Fig. 1). The time sequence of N_2O fields in Fig. 2 shows evolution of the tongue over a five-day period: stretching of the tongue into middle latitudes is followed by the breaking off of a patch of high- N_2O air near 40° S on September 10. This isolated patch of high- N_2O air retains its identity in middle latitudes until at least September 20, when the satellite yaws into a north-looking configuration.

Figure 3a shows a map of the 1,100 K water vapour distribution on September 8, obtained from MLS measurements. Water is also a long-lived tracer in the lower and middle stratosphere, with relatively low values in the tropics related to the upward tropical circulation (Fig. 1), and freeze-out near the cold tropical tropopause¹⁴; the mean structure is similar to that of N_2O , but with high and low values inverted. The water distribution in Fig. 3a shows a tongue-like structure very similar to that seen in the N_2O data, with relatively dry air extending from the tropics to midlatitudes. In a similar manner to the N_2O evolution seen in Fig. 2, this tongue of dry air is observed to stretch and break, with a net transport of dry air from the tropics to midlatitudes. Although the overall budgets of these tracers have not been

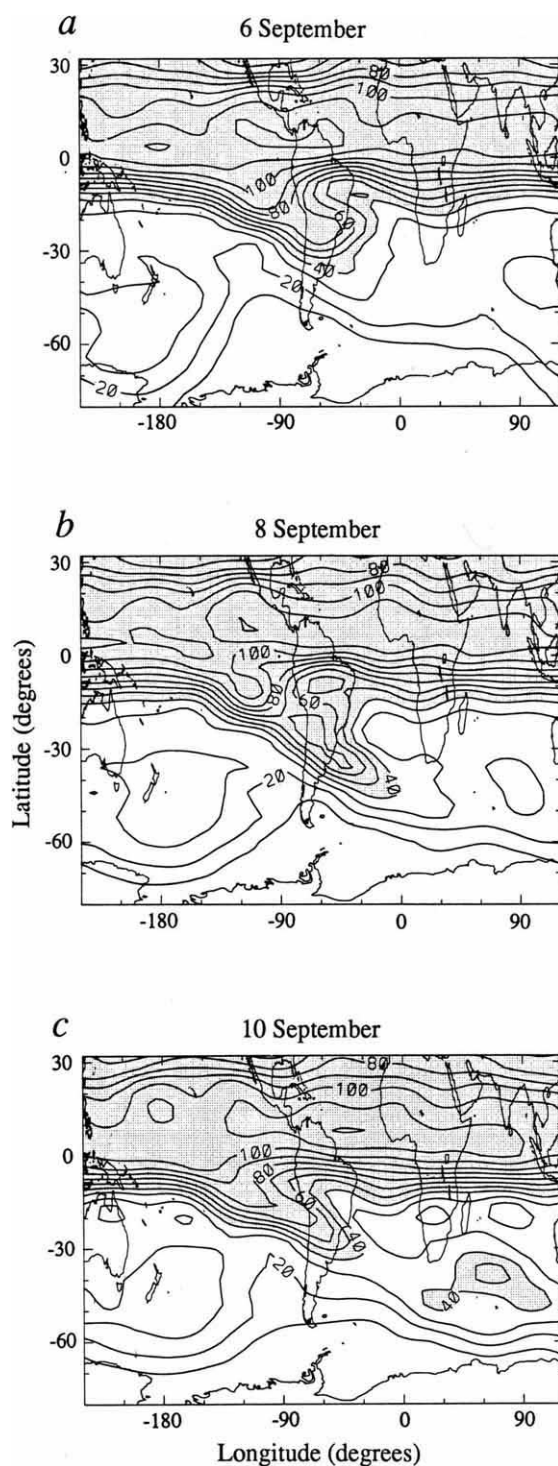


FIG. 2 a-c, Sequence of near-global maps of nitrous oxide (N_2O) mixing ratio (p.p.b.v.) on the 1,100 K isentropic surface for 6-10 September 1992. This isentropic level is near an altitude of 38 km, and close to a pressure surface of 5 hPa.

quantified, such transports out of the tropics are a possible factor contributing to the dehydrated midlatitude stratosphere observed over the Southern Hemisphere in October¹⁵.

Figure 3b shows a map of potential vorticity (PV) on the 1,100 K isentrope for 8 September. Potential vorticity is a conservative quantity often used for diagnosis of atmospheric fluid dynamics, in particular for variations associated with planetary-wave transience and mixing^{7-8,16-18}. Here PV is calculated by

combining MLS temperature data with base-level geopotential heights from the US National Meteorological Center (NMC), and then deriving balanced winds and potential vorticity¹⁹; the PV data are truncated to the same resolution as that used for the constituent data. The PV patterns in Fig. 3b show strong gradients in high latitudes (identifying the polar vortex), and a secondary region of tightened contours near 10°–20° S (the subtropical PV barrier). The PV structure shows clearly a tongue of low- (tropical-) PV air extending into midlatitudes, and the similar patterns seen in three completely independent quantities (N_2O , H_2O and PV) are strong evidence for the reality of this feature. A sequence of PV maps (not shown) reveals that the drawing out of this subtropical tongue occurs subsequent to a planetary wave deformation of the polar vortex (note the asymmetry of the vortex in Fig. 3b); as the vortex is deformed towards low latitudes, the associated winds advect material out of the tropics into middle latitudes (this is shown clearly in numerical simulations of this event²¹). Material exchanges between high and low latitudes are evidenced in these data by the separation (breaking) of PV and tracer contours; this irreversible exchange due to large scale motions is what we refer to as planetary wave mixing. We note, however, that the overall material exchanges are viewed somewhat differently depending on the background gradients: the PV structure highlights exchanges between the polar vortex and midlatitudes (note the polar PV remnants west of South America in Fig. 3b), whereas tropical-midlatitude inter-

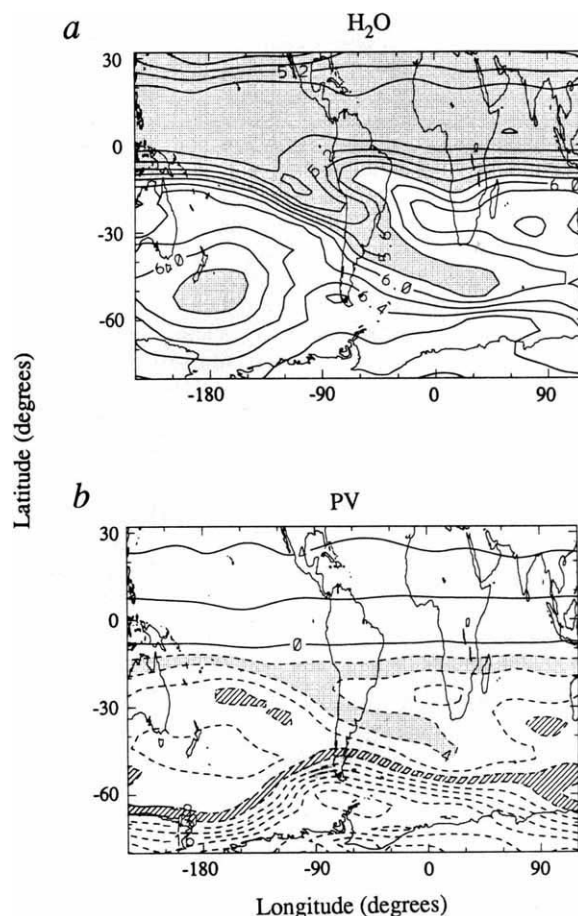


FIG. 3 Maps of water vapour mixing ratio (a, units of p.p.m.v.) and potential vorticity (b, units of $10^{-4} \text{ K m}^2 \text{ kg}^{-1} \text{ s}^{-1}$) on the 1,100 K isentropic surface on 8 September 1992. Water vapour data are from the MLS instrument on UARS, whereas potential vorticity is derived from MLS temperature and NMC base-level geopotential data (see text). Note the similarity in spatial structure between these data and the CLAES-derived nitrous oxide (Fig. 2b).

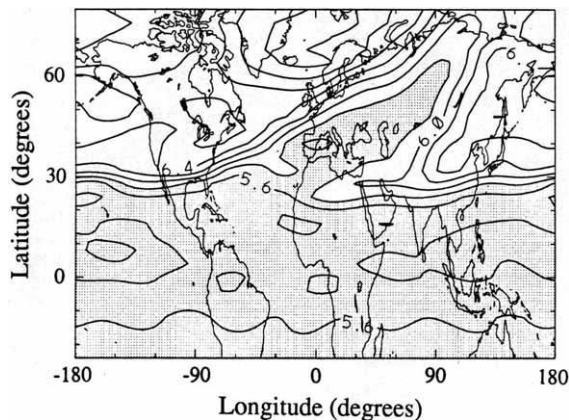


FIG. 4 Map of water vapour mixing ratio (p.p.m.v.) on the 1,100 K isentropic surface on 16 February 1993.

actions are emphasized in the constituent data (for example, the tropical N_2O values south east of Africa in Fig. 2c).

Figure 4 shows a diagram of a similar tongue-like structure in the distribution of water vapour for a wave event during Northern Hemisphere winter. A sequence of such maps (not shown) reveals separation of contours and the implied mixing of subtropical air into middle and high latitudes; note that the latitudinal excursions are larger for this case than for that in Figs. 2 and 3, as planetary wave amplitudes are typically larger in the Northern Hemisphere stratosphere. The evidence of wave transports out of low latitudes are most apparent in these constituent data during late winter-spring in both hemispheres; this time period is highlighted because the background tracer structure varies substantially with the seasonal circulation, with strongest subtropical gradients observed during late winter-spring.

The impression gained from the tracer measurements described here is that the subtropical transports appear mostly unidirectional—that is, material moves from the tropics to mid-

latitudes but not vice versa. This behaviour is similar to planetary-wave erosion of the polar winter vortices, where material is pulled off the outer edge and mixed into midlatitudes, but little transport into the vortex is found¹⁷. High-resolution simulations indicate that much of the erosion of the polar vortices occurs in the form of narrow filamentary structures, which cannot be observed in coarse-grain satellite data (but which are seen in aircraft observations)²⁰. Similarly, it is possible that erosion of the subtropical barrier also occurs via filamentation, in addition to the large-scale planetary waves observed here. Further analyses of UARS constituent data will allow insight into transport mechanisms and couplings between high and low latitudes; numerical simulations²¹ clearly show strong coupling between the low latitude perturbations shown here and planetary wave deformations of the polar vortex. □

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Subtropical stratospheric mixing linked to disturbances in the polar vortices

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RANDEL *et al.*¹ have observed tongues of stratospheric air stretching from the tropics into middle latitudes, and conclude that such events may be responsible for transporting significant amounts of stratospheric air across the tropical-mid-latitude barrier². Here I examine the movements of air parcels during these events using high-resolution contour-trajectory calculations. My calculations suggest that the tongues of tropical air are associated with disturbances of the stratospheric polar vortices. The edge of the disturbed polar vortex reaches low latitudes, and draws a long tongue of tropical air around the vortex into middle latitudes. This process occurs in the winter of both hemispheres, although the edge of the larger Antarctic polar vortex reaches farther toward the Equator, and draws up material from lower latitudes, than its Arctic counterpart.

Measurements from the recently launched Upper Atmosphere Research Satellite (UARS) are providing valuable insights into the distribution of trace constituents and fluid motions throughout the middle atmosphere. Randel *et al.*¹ have recently analysed global measurements of the long-lived stratospheric tracers nitrous oxide (N_2O) and water vapour (H_2O), which are measured by the Cryogenic Limb Array Etalon Sounder (CLAES) and the Microwave Limb Sounder (MLS) instruments on UARS, respectively. This analysis has shown that there are strong meridional gradients at low latitudes in the middle and upper stratosphere (altitude 28–42 km). The existence of these strong gradients confirms earlier suggestions² that there is a barrier to quasi-horizontal transport from the tropics into middle latitudes. But Randel *et al.*¹ also showed events where tongues of tropical air extend deep into middle latitudes. These tongues stretch and appear to break into isolated 'patches', suggesting that there is irreversible mixing and transport of tropical air into middle latitudes. This apparent subtropical wave breaking is analogous to wave breaking at the edge of the polar vortex^{3,5}. Tongues of tropical air have also been detected in previous observational data⁵, and in numerical models^{7,17}.

Here we examine the formation of these tongues of tropical air using 'contour advection with surgery' (CAS) calculations (this technique is described by Waugh and Plumb⁶; similar approaches have been used by Norton⁷ and Schoeberl *et al.*⁸). In CAS calculations the evolution of material contours on a given isentropic surface is determined by advecting the contours

by the daily, analysed balanced winds on that surface (as calculated from stratospheric analyses provided by the National Meteorological Center (NMC)). The resolution of the contours is maintained as they evolve, and very small-scale features are eliminated, using 'contour surgery' procedures⁹. Comparisons of CAS calculations with aircraft observations of chemical tracers^{10,11} have shown that these calculations can accurately resolve fine-scale features (much smaller than those resolvable by operational satellites) in the stratospheric transport of tracers.

During 6–10 September 1992 a tongue of tropical, upper-stratospheric air was observed (in both the N_2O and H_2O data) to extend into southern middle latitudes (see Figs 2 and 3 in ref. 1). Fig. 1 shows the results from a CAS calculation on the 1,100 K isentropic surface (altitude 38 km) for this period. The material-contour calculation was started on 1 September at the 15° S latitude circle (this is in the region of strong gradients observed by UARS, see Fig. 1 of ref. 1). There is excellent agreement between the CAS calculation and the observed tracer distributions (Figs 2 and 3 in ref. 1). A tongue of tropical air extends over South America into middle latitudes and this tongue then stretches out and forms a 'patch' of tropical air over the southern Indian Ocean. As in previous comparisons^{10,11}, the CAS calculation produces fine-scale filamentary features not detectable in the satellite data, for example the thin strands of tropical air joining the 'patch' over the southern Indian Ocean to lower latitudes. These strands narrow and lengthen with time, eventually reaching scales where diffusive processes mix the tropical and middle latitude air. The good agreement between the CAS calculations and the UARS data implies that the balanced winds derived from NMC analyses accurately describe the tropical flow field.

Randel *et al.*¹ noted that the intrusions of tropical air into middle latitudes can also be detected in maps of Ertel potential vorticity (PV) on the 1,100 K isentropic surface. Potential vorticity is a materially conserved quantity for adiabatic, frictionless flows, and provides a powerful diagnostic indicator of the fluid motions^{12,13}. Maps of PV for 6–10 September 1992 also show strong deformations to southern high-latitude contours (for example Fig. 3 of ref. 1), which suggests that there is also transport from the edge of the polar vortex into middle latitudes. The high-latitude tracer transport, and interaction with low latitudes, is now examined using CAS calculations started with contours of PV (calculated from NMC analyses).

Figure 2 shows the evolution, from a CAS calculation, of two material contours: the dark-shaded inner contour was initially at the outer edge of the Antarctic polar vortex, whereas the light-shaded outer contour was initially at the southern edge of the region of strong gradients in the tropics. These maps are polar stereographic projections, with South America at the top of the map. The tongue of tropical air shown in Fig. 1 now appears as a thin (lightly-shaded) ribbon on the right side of the map, which by 10 September extends from the top to the bottom of the map. The time sequence of these plots suggests that this tongue of tropical air is produced by the movement of the polar vortex to lower latitudes. As the polar vortex moved off the pole, the outer edge reached low latitudes (for example, 30° S over South America on 6 September) and draws tropical air to higher latitudes. This tropical air formed a thin tongue which was stretched and wrapped around the vortex. The calculation also shows that during this period tongues of air from the outer edge of the polar vortex are mixed into middle latitudes. This transport of material, away from the edge of the vortex in long thin filamentary tongues, is characteristic of planetary-scale Rossby wave-breaking at the edge of the polar vortex in the middle and upper stratosphere^{3–5}. The filamentary structures in middle latitudes can not be detected in satellite data, but have been detected in aircraft observations^{10,11,14,15}.

The tongue of tropical air has very deep vertical structure, and is present in the lower stratosphere (for example, 450 K isentropic surface, altitude 17 km). The tongue was not detected

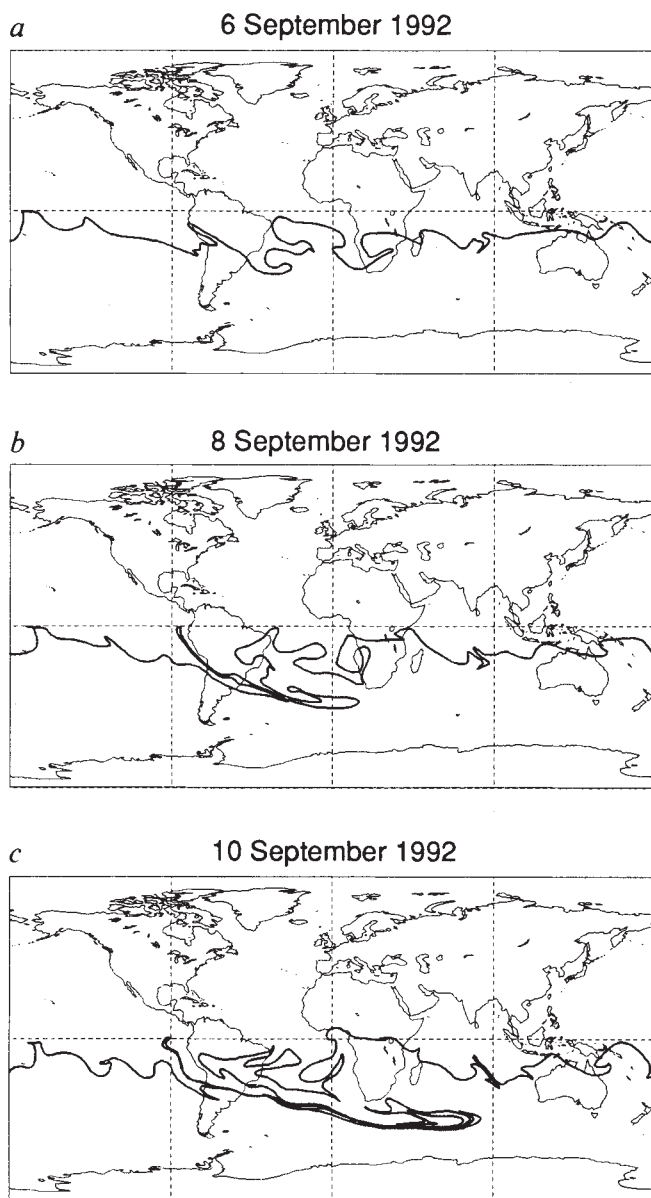


FIG. 1 a, b, c, Evolution of a material contour on the 1,100 K isentropic surface (approximate altitude 38 km and pressure 5 mb) for 6–10 September 1992, as determined by a CAS calculation. The calculation was started on 1 September 1992 at the 15° S latitude circle.

in the UARS data at the lower levels, presumably because the trace constituents do not have strong gradients at these levels¹. But satellite measurements of aerosols (taken soon after the eruption of Mount Pinatubo) show a tongue of tropical, lower-stratospheric air penetrating into southern middle latitudes¹⁶. CAS calculations on the 500 K isentropic surface for this period show very similar evolution to that described above, again suggesting that the formation of the tropical tongue was associated with the movement of the edge of the polar vortex.

The evolution described above also occurs in the Northern Hemisphere winter. Figure 3 shows the position of two material contours on 16 February 1993 from a CAS calculation on the 1,100 K isentropic surface started on 10 February. The inner contour was initially at the outer edge of the Arctic vortex whereas the outer contour was at the northern edge of the strong gradients in the tropics. As in the Southern Hemisphere case,

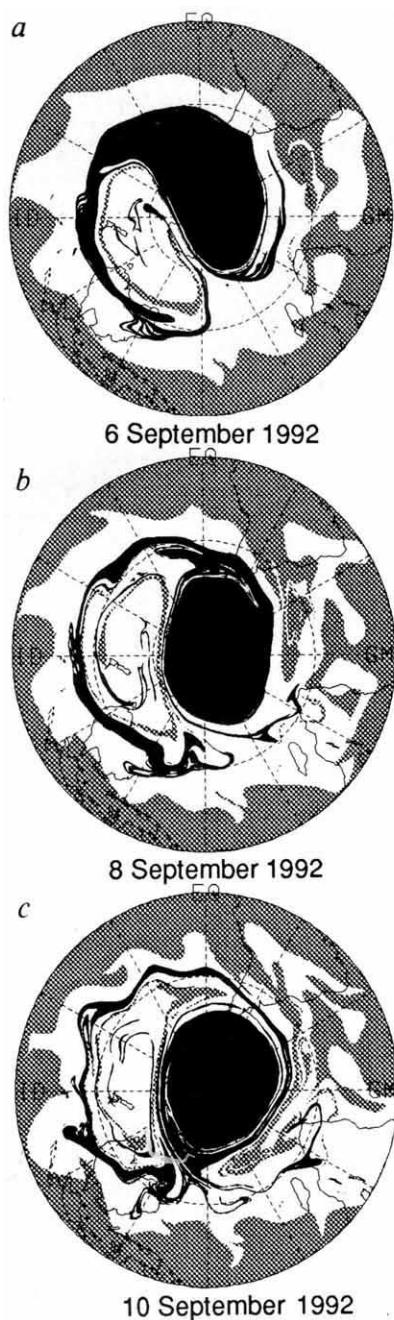


FIG. 2 *a, b, c*, Evolution of material contours at the edge of the polar vortex (dark-shaded region) and the strong gradients in the tropics (light shading) on the 1,100 K isentropic surface for 6–10 September 1992, as determined by a CAS calculation. The initial contours are at the analysed locations of the -7×10^{-4} and $-3 \times 10^{-4} \text{ K m}^2 \text{ s}^{-1} \text{ kg}^{-1}$ contours of Ertel PV (see text) on the 1,100 K surface on 1 September 1992. Maps are polar stereographic projections with the Greenwich meridian to the right.

the polar vortex is distorted into a comma-shape and is centred away from the pole. The time sequence of maps shows the edge of the vortex reaching low latitudes and drawing a tongue of tropical air over Europe and around the vortex. The structure and position of this tongue on 16 February agree well with the H_2O observations from UARS (see Fig. 4 of ref. 1). Again, there are also breaking Rossby waves at the edge of Arctic vortex,

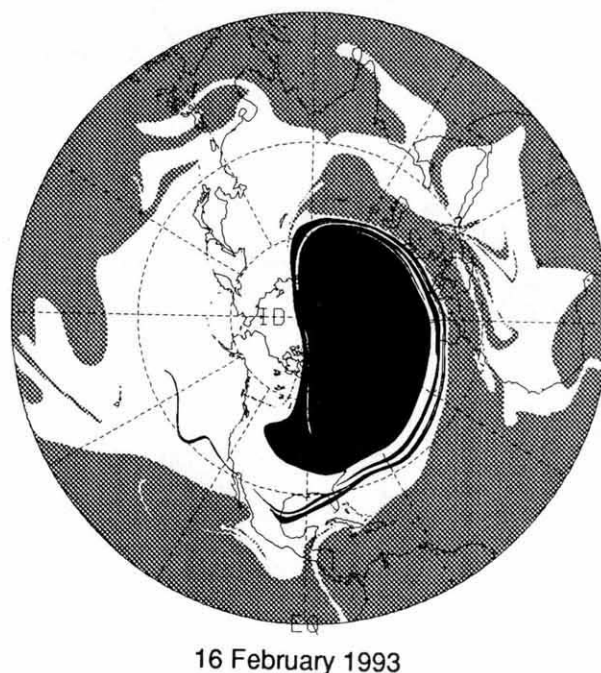


FIG. 3 Position of contours at the edge of the polar vortex (dark-shaded contour) and the strong gradients in the tropics (light shading) on the 1,100 K isentropic surface on 16 February 1993, as determined by a CAS calculation started on 10 February. The initial contours are at the analysed locations of the 8×10^{-4} and $4 \times 10^{-4} \text{ K m}^2 \text{ s}^{-1} \text{ kg}^{-1}$ contours of Ertel PV (see text). Map is a polar stereographic projection with the Greenwich meridian to the right.

and thin tongues of both polar and tropical air are transported (and mixed) into middle latitudes.

Although the evolution during the above periods is similar, the edge of the larger, although less mobile, Antarctic polar vortex reaches further towards the Equator than its Arctic counterpart, and material from lower latitudes is mixed into southern middle latitudes. Hence the extent of movement of the edge of the polar vortex may explain the hemispheric difference between the observed position of the region of strong gradients in the tropics (the strong gradients of N_2O in the Northern Hemisphere winter are at $20\text{--}30^\circ \text{ N}$ compared with $5\text{--}15^\circ \text{ S}$ in the Southern Hemisphere winter¹). □

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Evidence for large pre-industrial perturbations of the Black Sea chemocline

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RECENT studies^{1–8} have documented significant short-term vertical fluctuations in the position of the oxic–anoxic interface (chemocline) in the waters of the Black Sea, the world's largest anoxic basin. Natural^{5,9} and anthropogenic^{3,4,8,10} influences have been invoked as possible causes of the observed fluctuations, but it has been difficult to establish the relative importance of these two forcings. One reason is that observations of the magnitude of chemocline displacement have not extended sufficiently far in the past to eliminate the possibility of anthropogenic changes in freshwater input. Here we present chemical analyses of shelf sediments, collected from the Bosphorus region of the Black Sea, which contain a record of past water column chemistry. We find that the chemocline in this region rose by at least 40–50 m $\geq$ 250–300 years ago, precluding anthropogenic forcing as a viable cause. Although our results do not rule out an anthropogenic cause for the recent variations, they do show that natural perturbations more than twice as large as the more recent changes have occurred in the past.

Sediment samples from the oxic zone of the Black Sea were collected along two box-coring transects on the Turkish outer shelf (Bosphorus region and Bay of Sinop) during leg 4 of the 1988 RV *Knorr* cruise¹¹ (Fig. 1). Suboxic diagenetic conditions¹² characterized by strong bioturbation, undetectable interstitial dissolved sulphide, low extents of bacterial sulphate reduction and elevated levels of pore-water dissolved iron were typical of all sediments. As expected from the suboxic pore-water conditions, the sediments at all subsurface depths at the Bay of Sinop (stations 16 and 17) and in the uppermost centimetres in the Bosphorus region (stations 3 and 4) were also characterized by low concentrations of reduced sulphur (≤ 0.25 wt%) and a low degree of sulphidation of reactive iron ($< 10\%$) (Figs 2 and 3). By contrast, sediments below the surface intervals at the two Bosphorus localities showed very high concentrations of reduced sulphur (mainly as pyrite), with midcore maxima of ~ 3.2 and 2.4 wt% sulphur at stations 3 and 4, respectively. These subsurface sediments also displayed high concentrations of solid reactive iron and a high degree of sulphidation of the reactive iron phases ($> 60\%$) (Fig. 3). The extreme sulphur enrichments (compared with marine sediments in general) and the high degree of sulphidation were not associated with appreciable increases in the levels of organic matter, suggesting that they do not reflect a period of enhanced diagenetic sulphate reduction¹³ (see ref. 14 for the effects of non-steady-state inputs of organic matter). In fact, these values exceed, by almost a factor of two, the highest levels of pyrite sulphur measured in the entire suite of Black Sea sediments collected during leg 4 from a wide range of depositional environments, including sites in the deep anoxic-sulphidic central basin¹⁵. The peaks of sulphur concentration correspond with high depositional inputs of iron (with the capacity to react to form pyrite¹⁶) far in excess of those associated with the normal detrital iron flux. Matching this was a high concentration of solid-phase manganese; values ranging up to 900–1,000 parts per million (p.p.m.) (stations 3 and 4) compared with ~ 300 p.p.m. in the 'normal' suboxic sediment (compare Figs 2 and 3).

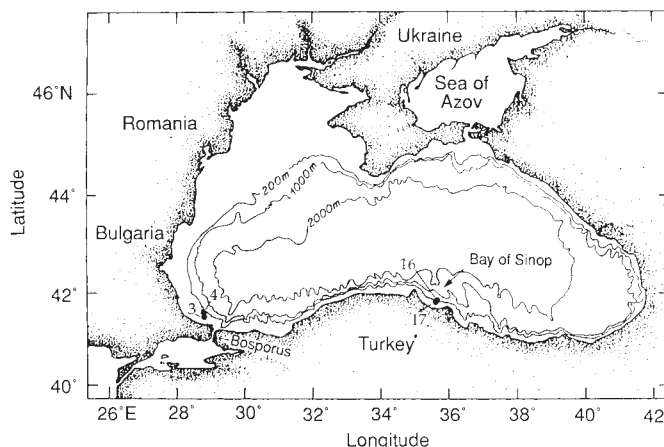


FIG. 1 Location map displaying the sediment sampling sites described in the present study. These cores of bioturbated, grey–brown shelly mud (including shell layers dominated by fragments of *Mytilus* at station 3) were collected during June and July (leg 4) of the 1988 RV *Knorr* expedition and represent oxic sites in the Bosphorus and Bay of Sinop regions sampled during two box-coring transects spanning the impingement of the present-day chemocline with the basin-margin substrate. Water depths at stations 3, 4, 16 and 17 are 85, 115, 129 and 97 m, respectively. Lyons¹¹ has provided additional details concerning the sample locations and the sedimentological characteristics of the box cores.

Pyrite (FeS_2) formation in sedimentary systems is usually controlled by any of three factors: (1) the availability of dissolved sulphate which is reduced via bacterial processes to H_2S , (2) the quantity and quality of the labile organic component used in this reduction and (3) the availability of reactive (towards hydrogen sulphide) iron^{13,17,18}. The invariant down-core trend for organic carbon at station 3 and the fact that the concentrations are similar to those found at the stations in the Bay of Sinop region indicate that supplies of organic carbon are not a factor in the development of iron-sulphide enrichments. Iron limitation is also not a significant consideration for the observed enrichments. Reactive iron (that is, iron easily extracted by chelating agents or HCl and therefore reactive towards hydrogen sulphide¹⁶) is plentiful both above and below the enriched layers, as well as in association with the sediments of the Bay of Sinop region. Without increased supplies of dissolved sulphide, elevated inputs of iron would not *a priori* lead to significant increases in the amount of pyritic sulphur. Further, high measured concentrations of dissolved sulphate in the interstitial waters of the sediments indicate that sulphate limitation can be discounted. The observed midcore solid-phase enrichments at stations 3 and 4 therefore cannot be attributed to ordinary marine conditions.

Given the impossibility of explaining the observed Fe–Mn–S enrichments at stations 3 and 4 by means of present-day conditions in the water column and ordinary diagenetic processes, we have interpreted our data as recording the intersection of the water-column Fe–Mn redox boundary with the outer-shelf bottom during a past chemocline (oxycline) shoaling event. This boundary is associated with a maximum in suspended iron and manganese oxyhydroxides^{19–22}; these phases were deposited on the shelf sediment bottom as a result of the shoaling. Further rise of the chemocline resulted in sulphidation of the iron oxyhydroxides (and a contribution from water-column pyrite formation) by anoxic–sulphidic bottom waters and preservation of the manganese enrichments likely as an insoluble carbonate phase. This represents perhaps the first well-documented example of manganese mineralization directly attributable to water-column, trace-metal redox cycling in a modern anoxic basin, an often-cited mechanism for generation of economic-grade manganese deposits in the geologic record^{23,24}. The high degrees of sulphidation of iron ($> 60\%$) observed in association with the midcore

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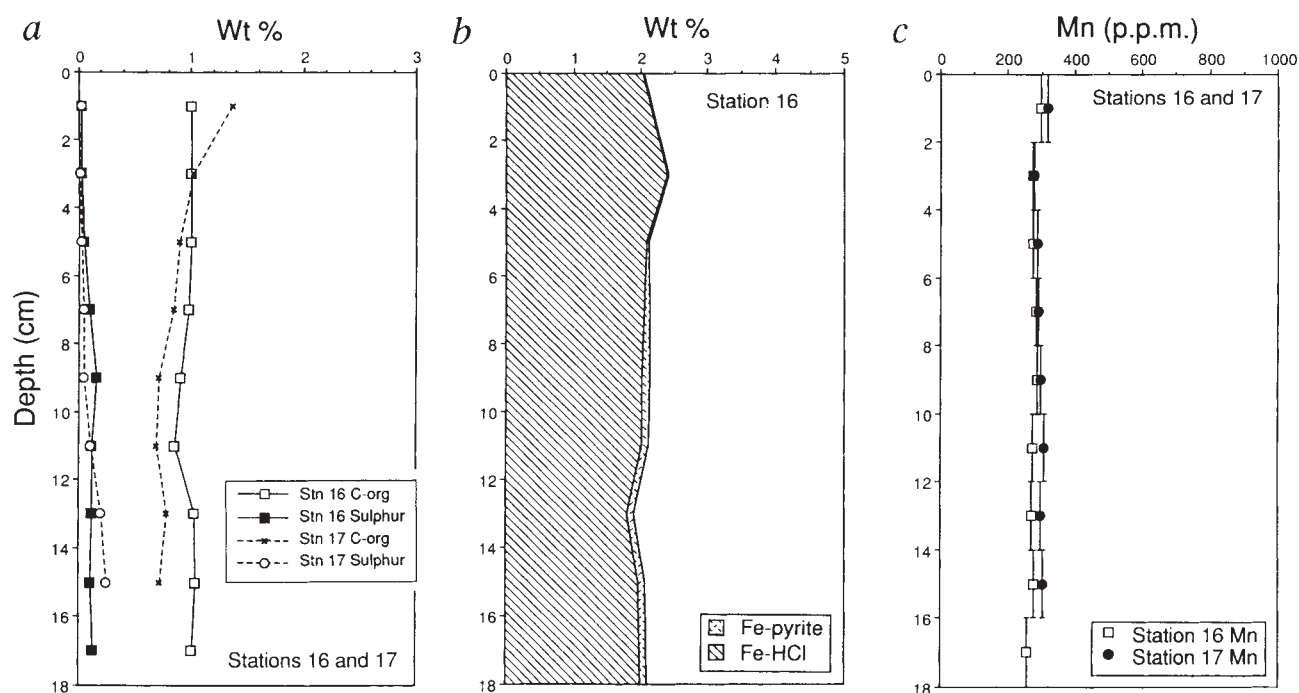


FIG. 2 Down-core distributions of solid-phase components at stations 16 and 17, oxic sites in the Bay of Sinop region. These results have been included to emphasize the contrast in Fe–Mn–S trends relative to the oxic Bosphorus sites (Fig. 3) despite strongly similar present-day (suboxic) diagenetic conditions. *a*, Organic carbon and total inorganic reduced sulphur²⁷ (pyrite S+elemental S+S in acid-volatile iron monosulphides) for stations 16 and 17 of the Bay of Sinop region. Below the upper several centimetres, these small amounts of reduced S are present predominantly as pyrite. *b*, Down-core distributions at station

16 for pyrite Fe and the fraction of solid-phase Fe readily solubilized during a boiling 12 M HCl digestion. The latter is assumed to approximate reactive Fe or, more specifically, that component of the solid-phase Fe reservoir with the potential to react with hydrogen sulphide^{15,25}. *c*, Levels of solid-phase Mn at stations 16 and 17 as extracted using 1 M hydroxylamine hydrochloride in 25% (v/v) acetic acid for 6 h^{28,29}. This method is thought to be specific for Mn bound in oxides, carbonates and, perhaps, some Mn from silicate minerals¹⁶.

sulphur maxima at stations 3 and 4 are diagnostic of anoxic–sulphidic (euxinic) deposition as indicated by numerous independent ‘degree of pyritization’ studies of the stratigraphic record²⁵.

Because the buried iron-, manganese- and sulphur-rich sediments are now located at a water depth as shallow as 85 m, a past (pre-anthropogenic) shoaling of the O₂–H₂S interface to more than ~40–50 m above the depth observed in the Bosphorus

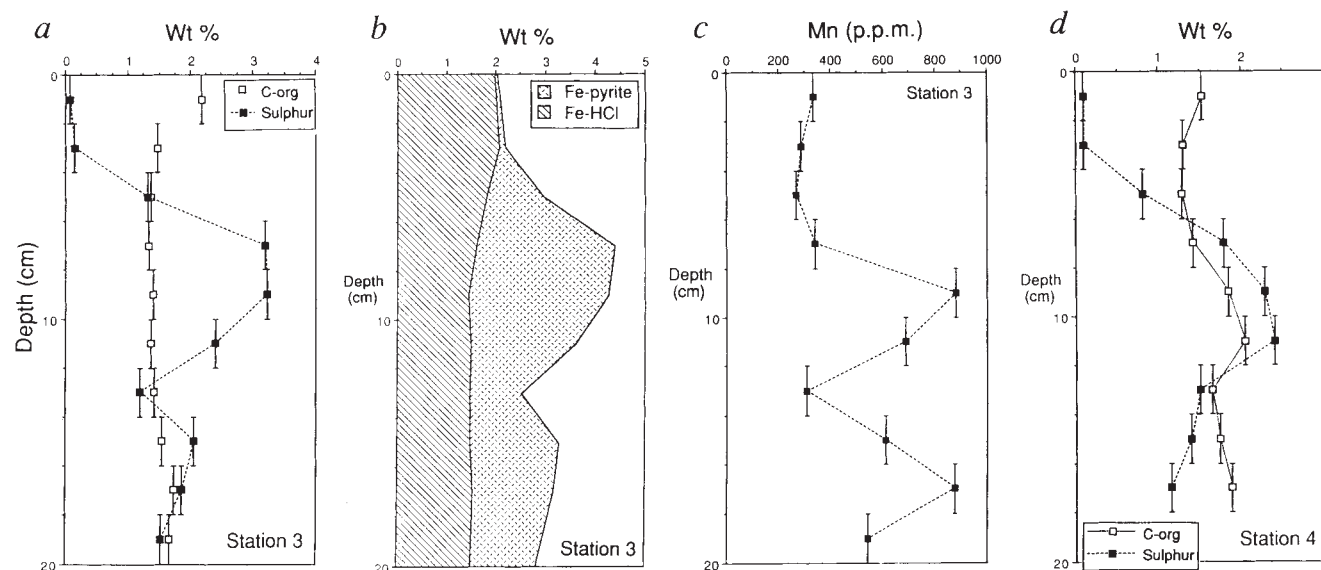


FIG. 3 *a*, Distributions with depth of organic carbon and inorganic reduced sulphur (present mostly as pyrite S) at station 3, an oxic site in the Bosphorus region. *b*, Depth profiles of pyrite Fe and HCl-extractable Fe at station 3. Procedural details are provided with Fig. 2. *c*, Down-

core distribution of solid-phase Mn at station 3 (see Fig. 2 for details). *d*, Distributions with depth of organic carbon and inorganic reduced sulphur (present mostly as pyrite S) at station 4, an oxic site in the Bosphorus region.

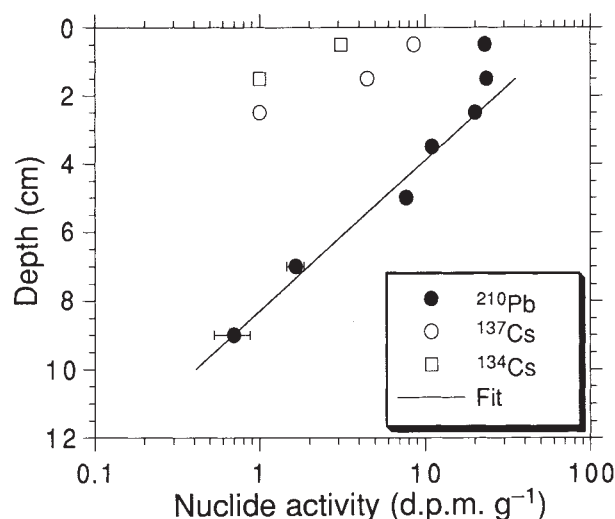


FIG. 4 Depth profiles of unsupported ^{210}Pb , ^{134}Cs and ^{137}Cs at station 3 (d.p.m., disintegrations per minute). Results for ^{210}Pb are from the present study; Cs activities are taken from ref. 30. An unweighted least-squares fit to the ^{210}Pb data between 2 and 10 cm gives an accumulation rate of 0.059 cm yr^{-1} . Activities of anthropogenic ^{134}Cs and ^{137}Cs were below detection at depths greater than $\sim 3 \text{ cm}$ at station 3. Detection limits for the Cs isotopes varied with sample size, but were typically a few tenths of d.p.m. g^{-1} .

region at the time of coring ($\sim 130 \text{ m}$) is indicated. Without additional coring at shallower depths, it is not possible to constrain the absolute upper limits of chemocline shoaling. It is reasonable, however, to infer a past injection of anoxic-sulphidic waters into the biologically active, shallow vertically mixed upper layers of the euphotic zone.

Unsupported ^{210}Pb activity measured at station 3 (Fig. 4) is interpreted to reflect a shallow ($\sim 2 \text{ cm}$) mixed layer overlying sediment accumulating at an average rate of 0.059 cm yr^{-1} . Distributions of anthropogenic ^{134}Cs and ^{137}Cs are consistent with this interpretation. Caesium-134, derived entirely from the Chernobyl accident in 1986, is confined to the mixed layer, whereas ^{137}Cs , derived both from the Chernobyl accident and from atmospheric testing of thermonuclear devices in the 1950s and 1960s, is detectable at slightly greater depth. Extrapolating the ^{210}Pb -based accumulation rate to the depth of the lower Fe-Mn-S peaks at station 3 suggests that the shoaling occurred $\sim 250\text{--}300$ years ago. The deeper enrichments mark the initial intersection of the rising chemocline with the sediments at station 3. Mixing of the sediments below a depth of $\sim 2 \text{ cm}$, although not recognized in the radionuclide data, would cause the apparent accumulation rate to exceed the true value; consequently, the inferred age of the shoaling event is a lower limit. Bioturbation after the proposed anoxic deposition at these presently oxic sites would have obscured any visual signal of anoxia (for example, sediment laminations). Bioturbation would also have the effect of broadening the chemical peaks observed in Fig. 3, thereby diluting the primary signature of the shoaling. With the restricted chronological details imposed by biological mixing, it is not possible to determine precisely the time and duration of the anoxic event in the Bosphorus region.

The Fe-Mn-S profiles of stations 3 and 4 are similar (Fig. 3)—enrichments induced by anoxic-sulphidic bottom waters at station 3 would be expected at station 4 (provided sediment accumulation rates are similar) given its close and slightly deeper position. The double-peaked nature of the enrichments at station 3 is thought to reflect small-scale chemocline depths fluctuations superimposed on a large-scale transgression of the chemocline. The spatial and temporal distributions and mechanistic details of this event, including the influence of the Bosphorus current system⁶, remain poorly constrained pending future coring along the Black Sea margin. The absence of enrichments in the box cores collected from the Bay of Sinop region suggests, however, that this phenomenon did not occur over all the basin. Our study demonstrates that the Black Sea water-column structure has

been dynamic on a scale of at least tens of metres during the past several centuries, thus lending credence to recent suppositions of present-day pycnocline/chemocline variability as a consequence of natural forcing⁵ (see also refs 9 and 26). In a broader sense, the results of our study reveal a geochemical signal with high preservation potential by which the dynamics of a palaeochemocline can be discerned. We hope these results will encourage further studies along the margins of anoxic basins, both modern and ancient. □

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Influence of the Earth's inner core on geomagnetic fluctuations and reversals

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In view of its relatively small size (one-third the radius of the outer core), many geodynamo models neglect the inner core entirely¹, or otherwise treat it as a non-conducting insulator^{2,3}. In a previous steady-state model⁴, we considered some effects of a finitely conducting inner core, in particular the resulting electromagnetic coupling between inner and outer core. Here we include a prescribed buoyancy force, which is geophysically more realistic, and also yields time-dependent rather than time-independent solutions. The field in the finitely conducting inner core does not then adjust instantaneously to the field in the outer core, but has a diffusive timescale of its own of a few thousand years. Rather large, rapid fluctuations in the outer core are then effectively averaged out by the inner core, producing a relatively stable external dipole field. We speculate that a geomagnetic reversal could only occur as a result of a particularly large fluctuation, large enough and lasting long enough to reverse the field throughout the inner core as well.

In our previous finitely conducting inner-core model⁴, we pointed out that the magnetic field in the inner core cannot be dealt with merely by imposing appropriate boundary conditions on the outer core, because it now has a diffusive timescale of its own, and is thus determined not only by changes on the boundary, but also by its own past history. But as the solutions we ultimately obtained were all steady-state, the potential presence of this new timescale was of rather limited significance. In this work it plays an important role. (There is another new timescale associated with the inner core, the inertial timescale of torsional oscillations. This timescale, however, is typically a few years⁵ rather than a few thousand years, and so we filter it out by neglecting inertia as before.)

Let the large-scale axisymmetric magnetic field be $\mathbf{B}_i$ in the inner core and $\mathbf{B}_o$ in the outer core, and let $\mathbf{U}$ be the large-scale axisymmetric fluid flow. Denoting the unit vector parallel to the rotation axis by $\hat{\mathbf{k}}$ and the radial vector by $\mathbf{r}$, the momentum equation in the outer core is then

$$2\hat{\mathbf{k}} \times \mathbf{U} = -\nabla p + E\nabla^2 \mathbf{U} + (\nabla \times \mathbf{B}_o) \times \mathbf{B}_o + \Theta \mathbf{r} \quad (1)$$

representing a balance of forces between the Coriolis (rotational) force on the left-hand side and, respectively, the gradient of the pressure p , viscous, Lorentz (magnetic), and buoyancy forces on the right-hand side. $\Theta = -\Theta_0 r \cos^2 \theta$ is the kinematically prescribed buoyancy force, chosen to drive a thermal wind essentially independent of the co-latitude θ . Considering that the geodynamo is ultimately driven by compositional or thermal buoyancy forcing, the inclusion of such a force is geophysically more realistic, although ideally the form of this force would also be dynamically determined rather than kinematically prescribed. The Ekman number E measures the ratio of viscous to Coriolis forces⁶. In the Earth's outer core, E is $0(10^{-12})$ or so, indicating that viscous forces are very small throughout the bulk of the core; we take E to be as small as is numerically possible⁷, which here unfortunately is no smaller than 10^{-3} , although this appears to be small enough to be in the asymptotic regime.

The mean-field induction equation is

$$\frac{\partial \mathbf{B}_i}{\partial t} = \nabla^2 \mathbf{B}_i \quad (2)$$

in the inner core, and

$$\frac{\partial \mathbf{B}_o}{\partial t} = \nabla^2 \mathbf{B}_o + \nabla \times (\alpha \mathbf{B}_o) + \nabla \times (\mathbf{U} \times \mathbf{B}_o) \quad (3)$$

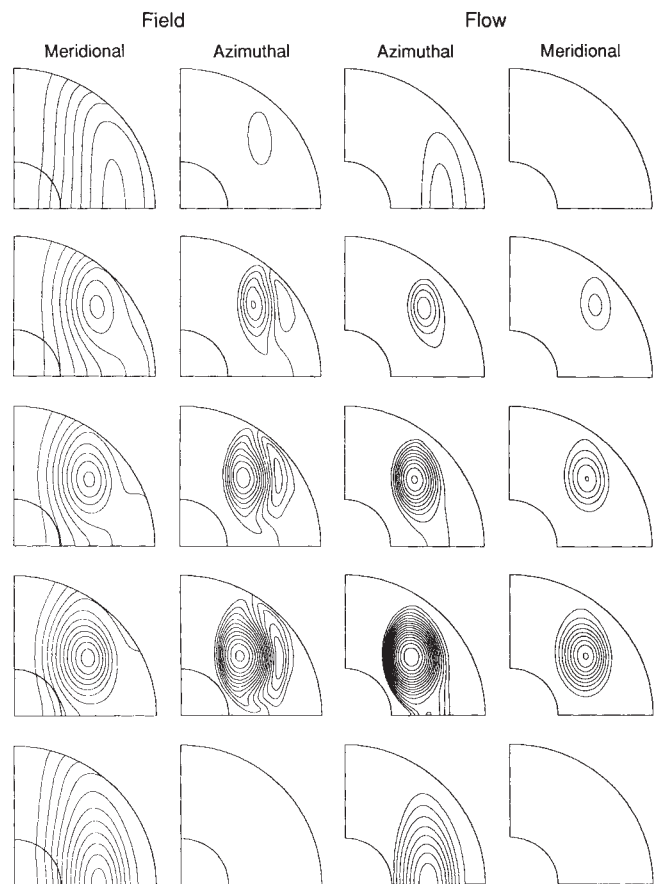


FIG. 1 From left to right, contour plots of the streamfunction of the meridional field, the azimuthal field, the azimuthal angular velocity, and the streamfunction of the meridional velocity for five (from top to bottom) uniformly spaced time intervals throughout the period of 0.22, ~13,000 yr in real time. Contour intervals of 1/2, 1, 50 and 5. The axis of rotation is vertical, and all quantities are axisymmetric about it. The ratio of inner- to outer-core radius, r_i/r_o , is 1/3.

in the outer core. The $\nabla^2 \mathbf{B}$ terms represent diffusion of the field throughout the entire core, $\nabla \times (\mathbf{U} \times \mathbf{B}_o)$ represents the inductive effects of the large-scale axisymmetric fluid flow in the outer core and $\nabla \times (\alpha \mathbf{B}_o)$ represents the (parameterized) inductive effects of the small-scale non-axisymmetric fluid flow in the outer core. The value of α , which is in some sense a measure of the departure of the flow from axisymmetry, was previously⁴ taken to be the scalar $\alpha_0 \cos \theta$; we retain the same spatial dependence but now take α to be a tensor by including only that component which regenerates meridional (in the planes of lines of longitude) from azimuthal (parallel to lines of latitude) field. The azimuthal field is thus not regenerated from the meridional by the α -effect, but by the above thermal wind by means of the so-called ω -effect⁶.

Fixing $\Theta_0 = 200$, and incrementally increasing α_0 , the onset of dynamo action occurs at $\alpha_0 \approx 8$, in the form of dynamo waves propagating from the equator to the pole. A secondary, symmetry-breaking bifurcation occurs at $\alpha_0 \approx 12$. The dynamo waves are then no longer oscillations about a zero time-average, but about a non-zero time-average. This bifurcation sequence corresponds exactly to the viscously limited solutions obtained previously¹ in the absence of an inner core, and so the presence of the inner core does not appear to be particularly significant at this stage.

A new and rather interesting type of solution does occur, however, if α_0 is further increased to $\alpha_0 \geq 40$. Figure 1 shows one period of the resulting solution at $\alpha_0 = 50$. Contour plots of the field and the flow are shown at five uniformly spaced time

intervals throughout the oscillation of period 0.22, or $\sim 13,000$ yr in real, dimensional time⁶. Note how virtually the entire dynamo process is confined to the region outside the inner-core tangent cylinder. We previously⁴ pointed out that in the limit of vanishing viscosity the field had to adjust to have a vanishing electromagnetic torque on the inner core (see also ref. 8), and that it seemed to do this by expelling the azimuthal field from the inner core. Here it seems to adjust by expelling the azimuthal field not just from the inner core, but from the entire region inside the inner-core tangent cylinder as well.

The solution shown in Fig. 1 starts out at the beginning of the periodic cycle with some meridional field and azimuthal flow, but essentially no azimuthal field or meridional flow. As time progresses, the azimuthal flow then gradually increases, and in the process draws out the meridional field to generate azimuthal field. The maximum amplitude of the azimuthal flow is quite large; one circuit in longitude is completed in $\sim 1,000$ yr, which is comparable to the observed westward drift^{9,10} (and indeed this azimuthal flow is in the westward direction). The azimuthal flow contours are closely aligned with the meridional field contours during most of the cycle, a feature also seen in model-Z dynamos¹¹. In consequence, the azimuthal flow is rather inefficient at drawing out the meridional field, so the azimuthal field strength is not simply the (large) magnetic Reynolds number times the meridional field strength. Because of this, our model has a smaller azimuthal field than has sometimes been postulated in the outer core⁶. Our model also shows a sudden collapse of field strength (again in about 1,000 yr, less than 1/10 of the total period), after which the cycle begins anew. This collapse appears to be due to the build-up of a misalignment of the meridional field and the azimuthal flow (perhaps due to the enhanced meridional flow); the magnetic tension associated with the meridional field then suddenly brakes the azimuthal flow.

So, we observe in Fig. 1 a periodic solution to the dynamo equations (1)–(3) that shows very substantial and occasionally very rapid fluctuations. If one now considers the only feature of this solution that would be directly observable in the real geodynamo, namely the external potential field (to which the meridional field matches) these fluctuations are not nearly so substantial. Figure 2 shows the (non-dimensional) dipole moment of this solution as a function of time; for comparison, the Earth's dipole moment in these units would be about 0.2, so the solution obtained here is unfortunately rather large. Although the solution within the outer core undergoes fluctuations of a full order of magnitude, at the core–mantle boundary its variation is considerably less. Note that the meridional field lines that emerge at the core–mantle boundary are largely the same field lines that also thread the inner core (note, for example, the bottom left panel of Fig. 1); that is, the structure of the field is such that the inner core and the mantle are more closely linked than one might think. This is the stabilizing role we envisage for the inner core; it has a diffusive timescale of its own which is comparable to the rapid fluctuations in the outer core, and effectively averages these fluctuations out to produce a much more stable external field than is typically obtained in models without an inner core¹. Indeed, fluctuations in the external dipole moment of the same relative magnitude (and occurring on the same $10\text{--}20 \times 10^3$ yr timescale as that obtained here), have been observed in the geomagnetic record¹².

To provide evidence that these solutions really are controlled by the inner core, a number of runs were also done with radius ratios (r_i/r_o), ranging from 1/4 to 1/2. The results show that as the location of the inner-core tangent cylinder changes with changing radius ratio, the dynamo process is consistently confined to the region just outside that cylinder. Figure 3 shows one timestep of the solution for a radius ratio of 1/2. At radius ratios of 1/4 or less, this solution ceases to exist. Not surprisingly, if the inner core becomes too small it can no longer control the dynamics. Finally, the fact that it is the location of the inner-core tangent cylinder that controls these solutions proves that

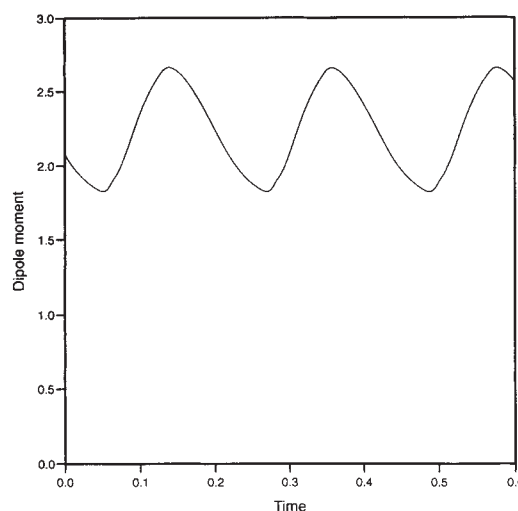


FIG. 2 The external dipole moment as a function of time. Again, the period of 0.22 is $\sim 13,000$ yr in real time, and the Earth's dipole moment in these units would be about 0.2.

rotational forces are considerably more important than viscous forces⁷, that is, that our value of 10^{-3} for the Ekman number is sufficiently small.

Considering that the geodynamo model presented here is only an axisymmetric, mean-field model, one should not read too much into the specific details. This model is not intended to be a precise, quantitatively accurate model of the geodynamo, and in view of the rather large external dipole moment it clearly is not. Nevertheless, it demonstrates quite clearly that the presence of the inner core can significantly influence the dynamo processes in the outer core. There is also direct observational evidence for this¹³; the magnetic flux at the core–mantle boundary in the polar regions is lower than that of a pure dipole, a feature that is produced by our results (Fig. 1).

Furthermore, the fact that one can obtain solutions showing such large fluctuations within the outer core, and still maintain a relatively stable external dipole moment, suggests the following plausible reversal mechanism: instead of an exactly periodic solution, as obtained here, imagine a quasi-periodic solution. If a larger than average fluctuation occurred, large enough and lasting long enough to reverse the field in the inner core, the whole field might reverse, but still be relatively stable both before and after. We have demonstrated here that the stabilizing effect of the inner core can prevent most fluctuations from leading to reversals, but if a particularly large fluctuation does lead to a reversal, the inner core will of course equally effectively stabilize

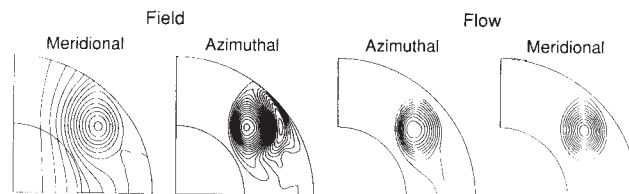


FIG. 3 Contour plots as in Fig. 1, but for an inner- to outer-core radius ratio of 1/2. The phase in the periodic oscillation is approximately at maximum—that is, roughly corresponding to the fourth row of panels in Fig. 1. The larger radius ratio causes the solution to be squeezed more strongly into the outer part of the spherical shell, consistent with the position of the inner-core tangent cylinder. Contour intervals as in Fig. 1.

the new, reversed state. The difficulty with many previous geodynamo models has been that virtually all fluctuations lead to reversals. This idea that reversals may be triggered by unusually large fluctuations has been proposed before^{14,15}; we have provided a more specific mechanism as to how it might function.

Finally, it should be pointed out that if, as widely believed^{16,17},

a stably stratified layer exists at the core-mantle boundary, that layer would tend to enhance this stabilizing averaging-out process. This could further reconcile the complicated, chaotic nature of the flow and field in the field-generating portion of the outer core with the much more stable nature of the externally observed dipole component of the main field. □

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Recent discoveries of *Dryopithecus* shed new light on evolution of great apes

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THE origin and early evolution of the great ape/human clade (Hominidae) is currently a subject of debate^{1–3}. The controversy is fuelled by the fragmentary nature of the fossils which renders it difficult to determine clearly derived features that permit the recognition of fossil members of this clade. We report here the recent discovery of a facial skeleton and a temporal fragment with the petrosal bone of *Dryopithecus laietanus*, which provides a way out of an impasse. The lack of the fossa subarcuata is a great ape and human clade synapomorphy, and proves unequivocally that *Dryopithecus* belongs to this clade. The zygomatic possesses derived characters which reveal that *Dryopithecus* is related to the Ponginae and not to the African apes/humans, as recently suggested¹. The remaining morphological features are plesiomorphic and thus provide a good model of a common ancestor of all Hominidae.

Dryopithecus is a middle and upper Miocene European hominoid first described from the French locality of St Gaudens in 1856 (ref. 4). The importance of this genus for the comprehension of the origin and evolution of the extant great apes is pertinent to the recent explosion of ideas about its phylogenetic position^{1–3}. Many of the arguments, however, have been based solely or primarily on dento-gnathic materials and very incomplete facial remains, which on their own seem to be inadequate for resolving the issues.

During the 1991 excavations at Can Llobateres (northeastern Spain), the type locality of the Vallesian Land Mammal Age, we recovered a series of cranial fragments of *Dryopithecus laietanus*. When reconstructed the fragments comprise the most complete specimen of the genus ever found (Fig. 1). This discovery thus provides a considerably more solid base to approach the problem of the early evolution of the great apes.

The well preserved petrosal bone is the only one known for early hominoids with the exception of *Proconsul*. Like all extant primates apart from the great apes and humans, *Proconsul*⁵ has a fossa subarcuata on the endocranial side of the petrosal⁶. In *Dryopithecus* it is absent (Fig. 2) and this is interpreted as a derived feature linking this genus with the clade of extant great apes and humans. Many of the supposed synapomorphies, for a long time used to prove the inclusion of fossil taxa in the great ape clade, were acquired independently as a result of adaptation

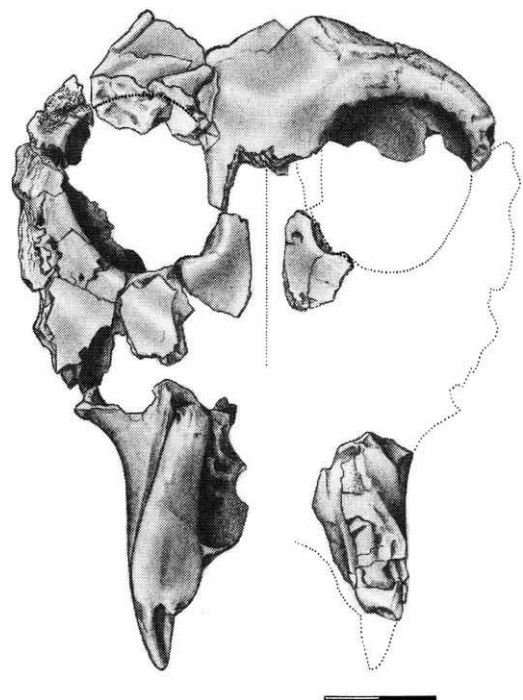
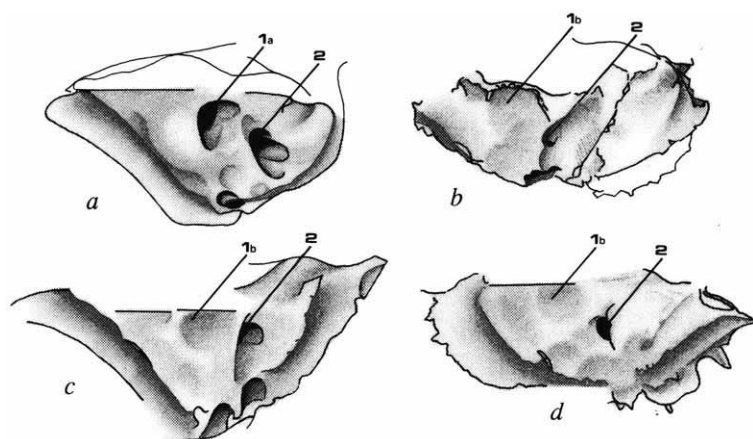


FIG. 1 The partial cranium CLL-18000 includes most of the frontal bone, the greater part of the right zygomatic, the two frontal processes of the maxillae, most of the right maxilla with canine to M³, the alveolar process of the left maxilla with P³ to M³, both the central incisors and a detached portion of the left temporal bone with the petrosal, the external auditory meatus and the mandibular fossa. As reconstructed the cranial fragments show a face which is broad at the orbital level. The orbits are separated by a broad interorbital pillar, are ovoid and slightly higher than wide. The frontal bone is not markedly elevated above the orbits, the zygomatic is strongly developed, broad and anteriorly facing. The alveolar process is high. Scale bar, 2 cm.

to environmental circumstances^{3,7}. Hence the presence of a synapomorphy found in an independent skeletal element of *Dryopithecus*, the complexity of which makes convergent acquisition of this character unlikely, is of great phylogenetic significance.

Starting from the inference that *Dryopithecus* is a member of the great apes, it can be interpreted further as either primitive with respect to all extant great apes and humans, and thus as the sister group of the latter, or it could pertain either to the clade of the African great apes and humans or to the *Pongo* clade. In the latter case it would have to share at least one apomorphic character with one of the two clades.

FIG. 2 Endocranial view of various hominoid petrosal bones a, *Hylobates*; b, *Dryopithecus laietanus* CLL-18000; c, *Pongo*; d, *Gorilla*; 1a, fossa subarcuata; 1b, cicatrix of fossa subarcuata; 2, internal acoustic meatus.



In general, *Dryopithecus* retains a lot of plesiomorphic characters. It is therefore thought to be a primitive hominoid and is taken as being positioned before the dichotomy between the African and Asian great apes^{3,8}. But several authors have suggested that it belongs to one or other of the later clades. Using the specimen RU 44 from Rudabanya (Hungary) as a basis it was considered¹ that the supraorbital morphology of *Dryopithecus* shows the derived condition of the African great apes and it was thus inferred that the klinorhynch (bending of the base of the skull) of *Dryopithecus* was the same as in the later clades. But for the supraorbital torus to be homologous to that of African apes two conditions must be fulfilled. First, it has to form a continuous bar of bone from one side of the face to the other, which includes the glabellar region. Second, there should be an extending of the ethmoidal sinus into the frontal to form a frontal sinus which occupies most of the supraorbital torus⁹. The Can Llobateres specimen does not comply with either of these requirements and neither, in our opinion, do the Rudabanya specimens. Despite the presence of a somewhat prominent glabella and a swelling of the part of the orbital rim which forms the supraorbital torus in *Dryopithecus*, there is no continuity between these two structures. In the Can Llobateres specimen a fracture of the frontal bone reveals that there is a well formed ethmoidal sinus which does not reach or penetrate the area of the torus or the orbital rim. We thus consider that this hypothesis can be rejected.

In contrast, the zygomatic bone of *Dryopithecus* shows what we consider to be derived morphology. Two characters stand out: (1) the overall robustness of the entire bone, which has a rugose superior part and (2) the presence of three prominent zygomaxillary foramina located high on its frontal process at about one third to a half the height of the orbit. Among the extant apes both of these characters can only be observed in

Pongo, in which they are functionally related to the presence of well developed cheek pads. The African apes possess the primitive morphology seen in *Hylobates* and *Proconsul*. We thus support the hypothesis that *Dryopithecus* is a primitive member of the *Pongo* clade¹⁰.

In this context, the interpretation of the fossil Eurasian apes is crucial for the construction of a plausible evolutionary model. *Sivapithecus* shares an important number of synapomorphies in the facial area with *Pongo*¹¹ and is thus a clear member of this clade. *Lufengpithecus* also shows some synapomorphies with the *Pongo* clade⁹. The position of *Graecopithecus* is more difficult to determine because of the poor condition of the zygomatic area. The robustness and the general morphology of this bone, the position of the temporal ridges well above the superior rim of the orbits and other characters, run counter to the suggestion that *Graecopithecus* is related to the African ape/human clade^{1,2,12}. Instead, we find that hypotheses linking *Graecopithecus* with the *Pongo* clade are more interesting. Our view of the phylogenetic relationships within the Hominoidea are summarized in the cladogram (Fig. 3).

If our phylogenetic interpretation is correct, a relatively simple biogeographic model emerges which explains the evolution and early radiation of the *Pongo* clade. During the middle Miocene, an unknown or unidentified African hominoid (which could be related to *Kenyapithecus*)³ dispersed outside Africa via the Iberian Peninsula towards Eastern Europe and thence to Asia. In this vast area, regional to local factors influenced evolution within the group and various morphotypes evolved. The most primitive forms, such as *Dryopithecus*, which are the most different from *Pongo* remained in Europe, whereas those reaching the Far East, such as *Lufengpithecus* and especially *Sivapithecus*, are morphologically and phylogenetically more closely

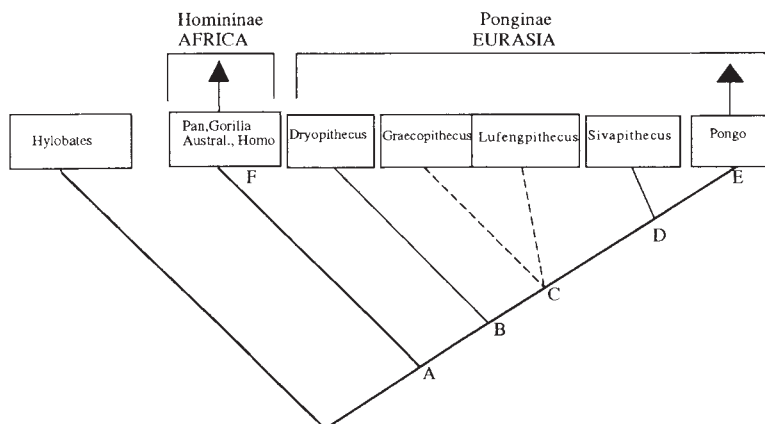


FIG. 3 Phylogenetic relationships of extant hominoids and fossil representatives of the *Pongo* clade. The nodes symbolize the following derived features or groups of characters: a, lack of the fossa subarcuata in the petrosal bone (see text); b, derived pongine zygomaxillary morphology (see text); c, derived pongine facial morphology (narrow interorbital pillar, airorhynch, lack of glabellar thickening, lack of fronto-ethmoidal sinus, high and narrow orbits^{1,3,10,11,13}); d, derived pongine dento-gnathic morphology (thick enamel, great size discrepancy between the upper incisors, alveolar prognathism and associated characters^{3,10,11,13}); e, derived postcranial anatomy of *Pongo* related to brachiation; f, facial klinorhynch, continuous supraorbital torus occupied by sinus, postcranial features related to knuckle walking^{1,3,13}.

related to extant *Pongo*. Meanwhile in Africa there remained a morphotype similar to *Dryopithecus* but with a primitive zygomorphic (like *Pan*), which could have given rise to the clade of extant African apes and humans, initiating the development of klinorhynch. But in the upper Miocene of Africa there exists an enormous fossil lacuna, which effectively renders the origin of the African ape/human clade matter of ongoing uncertainty. □

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A role for central vasopressin in pair bonding in monogamous prairie voles

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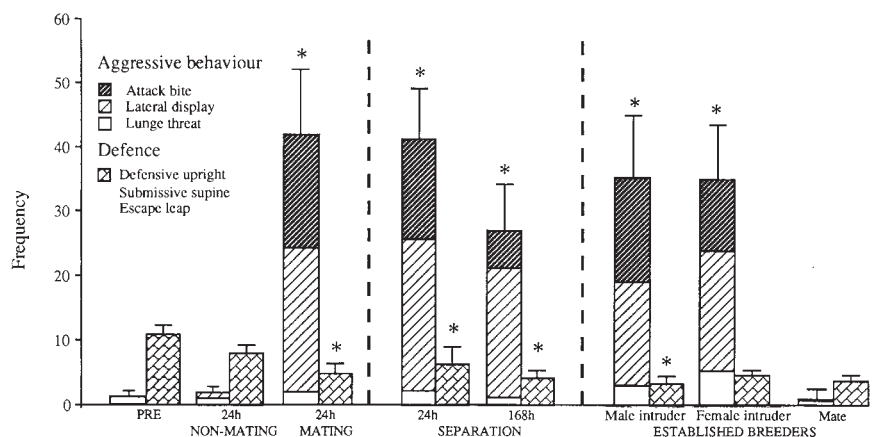
MONOGAMOUS social organization is characterized by selective affiliation with a partner, high levels of paternal behaviour and, in many species, intense aggression towards strangers for defence of territory, nest and mate^{1,2}. Although much has been written about the evolutionary causes of monogamy, little is known about the proximate mechanisms for pair bonding in monogamous mammals^{2,3}. The prairie vole, *Microtus ochrogaster*, is a monogamous, biparental rodent which exhibits long-term pair bonds characterized by selective affiliation (partner preference) and

aggression^{4,5}. Here we describe the rapid development of both selective aggression and partner preferences following mating in the male of this species. We hypothesized that either arginine-vasopressin (AVP) or oxytocin (OT), two nine-amino-acid neuropeptides with diverse forebrain projections, could mediate the development of selective aggression and affiliation. This hypothesis was based on the following observations: (1) monogamous and polygamous voles differ specifically in the distribution of forebrain AVP and OT receptors^{6,7}; (2) AVP innervation in the prairie vole brain is sexually dimorphic and important for paternal behaviour⁸; (3) central AVP pathways have been previously implicated in territorial displays and social memory^{9,10}; and (4) central OT pathways have been previously implicated in affiliative behaviours¹¹. We now demonstrate that central AVP is both necessary and sufficient for selective aggression and partner preference formation, two critical features of pair bonding in the monogamous prairie vole.

To characterize the development of selective aggression in prairie voles, sexually naive adult males were tested in a resident-intruder paradigm similar to that used with mice¹². Naive males, that is, males without female exposure, explored a novel male intruder but showed little attack behaviour. Within 24 h of mating, males showed a qualitative change in their behaviour towards an intruder, with vigorous attacks and threats and decreased defensive behaviour (Fig. 1). This increase in aggression seemed to be sustained and selective. Either with or without further exposure to a female, males continued to show attack

FIG. 1 Mean ($\pm$ s.e.m.) frequency of aggressive and defensive behaviour in 6-min resident-intruder tests. Before exposure to a female (Pre), sexually naive prairie vole males ($n=17$) exhibit minimal aggressive and moderate defensive behaviour towards a novel male intruder. Tested 24 h later, a subset of these same males ($n=8$) show a similar response to an intruder if housed in the interim with a female without mating (Non-mating). Another subset ($n=9$) that mated with females (Mating) exhibit a significant increase in aggression ($F(6,48)=7.44$, $P<0.05$) when tested after 24 h with the female (Dunnett's t -test, $t_D=3.97$, $P<0.05$) or after subsequent separation from their mates for 24 h ($t_D=4.10$, $P<0.05$) or 168 h ($t_D=2.61$, $P<0.05$). These same males respond to an intruder with comparable levels of aggression after being reunited with their mates for at least 1 week (Established breeder) ($t_D=3.28$, $P<0.05$). Aggression seems to be selective in that it is directed at both males and novel females, but rarely at the mate. A concurrent decrease in defensive behaviour is observed post-mating ($F(6,48)=3.89$, $P<0.05$). An asterisk signifies difference ($P<0.05$) from baseline frequency (Pre) in composite score of aggressive or defensive behaviour by within-subjects *post-hoc* Dunnett's t -test.

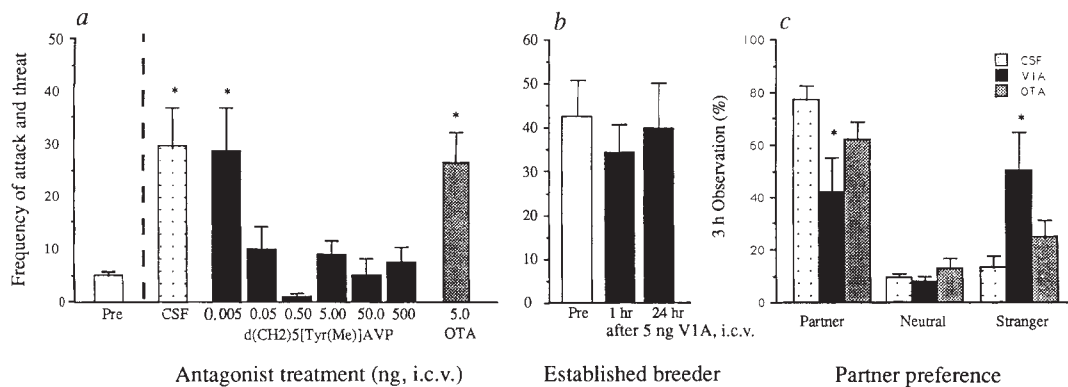
METHODS. For aggression testing, socially reared males (60–75 days old) were isolated for at least 3 days to avoid potential effects of social status in communal cages. For the resident-intruder model, an intruder



male was placed in the home cage of the experimental animal for 6 min. Each male was tested at baseline and then tested with a different intruder for subsequent tests. For groups with female exposure, the female was removed before adding the intruder male. Behaviour was recorded on videotape and scored using a computer-assisted data acquisition system by a rater blind to group assignment. Behaviour was operationally defined as previously described²² with reported means indicating frequency over the 6-min test.

FIG. 2 A V_{1a} antagonist given i.c.v. before mating blocked the induction of aggression in the male prairie vole. a, Before female exposure, males showed low levels of aggression (attacks and threats) in the resident-intruder paradigm as described for Fig. 1. Aggression increased after 24 h with a sexually receptive female if the male received i.c.v. injection of CSF, the OT antagonist OTA, or the lowest dose (5 pg) of V_{1a} antagonist $d(CH_2)_5[Tyr(Me)]AVP$ ($F(7,54) = 4.68$, $P = 0.0004$; CSF: Newman-Keuls *post-hoc* comparison, $q_7 = 8.97$, $P < 0.05$; 5 pg OTA: $q_7 = 8.25$, $P < 0.05$; 5 pg V_{1a} : $q_7 = 8.33$, $P < 0.05$). Males receiving all other doses of antagonist failed to increase aggressive behaviour above baseline frequency. Antagonist injection did not alter the number of males mounting females in the first 6 h ($\chi^2(d.f. = 7) = 2.186$, $P = 0.945$; range = 62.5–83.3%) or frequency of mounts ($F(3,19) = 0.82$, $P = 0.50$). b, When given to breeder males with established aggression at baseline, $d(CH_2)_5[Tyr(Me)]AVP$ (5.0 ng, i.c.v.) had no effect on the number of attacks or threats ($F(2,20) = 0.227$, $P = 0.80$). c, After 24 h of mating, a partner preference was observed in males injected with either CSF ($t = 6.71$, $P = 0.0005$) or OTA ($t = 3.061$, $P = 0.03$), but not in $d(CH_2)_5[Tyr(Me)]AVP$ ($t = 0.29$, NS) injected males. Treatment groups differed in time with the partner ($F(2,16) = 4.09$, $P = 0.04$) and time with the stranger ($F(2,16) = 4.21$, $P = 0.03$) due, in each case, to significant differences between the $d(CH_2)_5[Tyr(Me)]AVP$ - and CSF-injected voles ($t_0 = 2.86$, $P < 0.05$ in each case). Groups did not differ in activity as measured by number of entries into each of the chambers ($F(2,16) = 0.54$, NS).

METHODS. Before exposure to sexually receptive females, sexually naive males were given a baseline intruder aggression test as described for Fig. 1. Each male then received a single i.c.v. injection of either artificial CSF (BioFluids, Inc., Rockville, MD; $n = 13$); the OT antagonist OTA (Peninsula; $n = 8$)²³, or the V_{1a} antagonist $d(CH_2)_5[Tyr(Me)]AVP$ (Peninsula; $n = 6$ –8 per dose)²⁴. *Ex vivo* studies after central injection of $d(CH_2)_5[Tyr(Me)]AVP$ (50 ng, i.c.v.) demonstrated persistent blockade



of AVP-receptor binding for 18 h in the vole brain (data available on request), a time course which agrees with previous behavioural data in hamsters²⁵, our own behavioural data in voles (see Fig. 3b), as well as the time course of OTA activity²⁶. Injections (2 μ l) were given to halothane-anaesthetized voles using a 30-G needle attached via polyethylene-20 tubing to a 10 μ l Hamilton syringe. Because the vole skull is lightly calcified, injections could be reliably given percutaneously by using a cranial band for placement over the lateral ventricle²⁷. To verify placement, each injection included 10% india ink (v/v) and staining of the entire ventricular system was required at necropsy for inclusion of behavioural data (<10% rejected). After the injection, males were given sexually receptive females (ovariectomized and injected subcutaneously with 1.0 μ g oestradiol benzoate for 2–4 days). Behaviour was recorded for the next 24 h with time-lapse videotaping. Females were then removed and males received a repeat intruder test. Aggression was scored as for Fig. 1. Reproductive behaviour scored from the time-lapse videotapes included latency to first mount and number of mounts. In an independent study, males ($n = 19$) were similarly injected with CSF, OTA or $d(CH_2)_5[Tyr(Me)]AVP$ just before mating, and about 24 h later they were assessed for partner preference as previously described¹³ by measuring time in partner's (that is, mate's), stranger's (novel sexually receptive female) or neutral (uninhabited) cage over 3 h using time-lapse videotaping with 12:1 reduction. Partner preference was assessed by a paired t-test comparing time in partner's versus time in stranger's cage for each treatment, followed by a between-subject's factorial ANOVA to compare treatment groups on each measure.

behaviour 1 week later (Fig. 1). Attacks against the mate were not observed either during or after the initial 24-h mating experience, although females other than the partner were attacked as intruders (Fig. 1). Sexually naive males rarely show offensive aggression towards a conspecific female.

Mating seemed to be critical to the rapid induction of aggression, as males within 24 h of social but not sexual experience rarely show attack behaviour (Fig. 1). Mating has previously been shown in the female to help the development of a partner preference, critical for pair bonding in this species¹³. The development of aggression, essential for mate guarding and nest defence, may also be associated with pair bonding. In support of this hypothesis, no similar transition to aggression was seen in males of the closely related montane vole (*Microtus montanus*) which does not pair bond¹⁴. Montane males exhibited few attacks and threats when tested serially ($n = 7$) before mating (9.7 ± 2.9), 24 h after mating (4.7 ± 1.6) or while living with a female as part of a breeding pair (5.8 ± 1.9).

To investigate a role for either AVP or OT in the induction of aggression, male prairie voles were injected intracerebroventricularly (i.c.v.) with either cerebrospinal fluid (CSF), the vasopressin-1a receptor (V_{1a}) antagonist [1-(β -mercapto- β , β -cyclopentamethylene propionic acid), 2-(O-methyl)tyrosine]-arginine-vasopressin ($d(CH_2)_5[Tyr(Me)]AVP$) or the OT receptor antagonist $d(CH_2)_5[Tyr(Me)_2, Thr^4, Tyr-NH_2]$ ornithine vasotocin (OTA) just before 24 h of exposure to a sexually receptive female. After either CSF or OTA injection, males showed mating-induced aggression (Fig. 1). Males injected with a wide range of V_{1a} antagonist doses failed to exhibit aggression after

24 h with a sexually receptive female (minimum effective dose 50.0 pg i.c.v.) (Fig. 2a). This failure could not be attributed to blockade of mating, as reproductive behaviour was unaffected by any dose of the antagonist (see Fig. 2 legend). Furthermore, the V_{1a} antagonist did not seem to be anti-aggressive *per se*. Breeder males with established selective aggression showed no decrease in attack or threat behaviour either 1 or 24 h after i.c.v. injection of the antagonist (5.0 ng) (Fig. 2b). Thus, AVP antagonism seemed to block the transition to aggression, not its expression.

To determine if the V_{1a} antagonist blocked the development of selective affiliation as well as aggression, males were tested for a partner preference after 24 h of mating. Injections and mating were as above, but after 24 h of mating each male was moved to the neutral cage of a three-chamber partner preference apparatus¹³ with access to two adjoining cages, one with the mate (partner), the other with a novel female. Each female was loosely tethered to restrict movement out of her own cage. Males injected with CSF or OTA (5 ng, i.c.v.) before mating showed a significant preference for the mate 24 h later (Fig. 2c). After V_{1a} antagonist injection (5 ng, i.c.v.), males spent as much time with the stranger as the mate.

These results imply that physiological actions of endogenous AVP are essential for the induction of selective aggression and partner preference. But in initial studies in which we administered AVP (5–500 ng) as a single i.c.v. injection to sexually naive males, we observed no increase in aggression (data not shown). As the effects of sociosexual experience seemed to be mediated over several hours and the clearance of centrally injected AVP

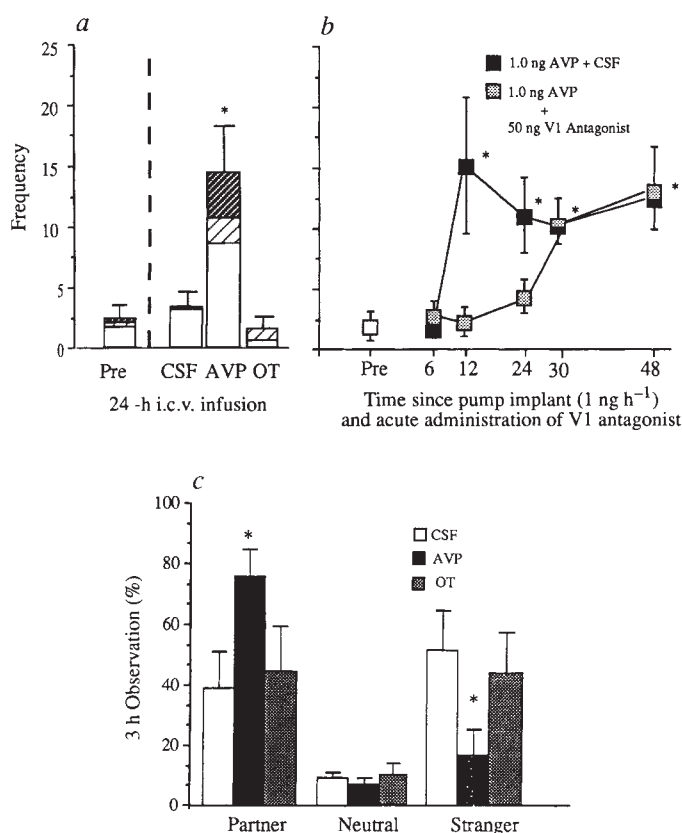


FIG. 3 Central infusion of AVP increases aggression and induces partner preference. **a**, After 24 h i.c.v. infusion of AVP (0.5 ng h^{-1}) but not CSF or OT (0.5 ng h^{-1}), aggression towards an intruder increases in male prairie voles. Groups did not differ before infusion (Pre), but in post-infusion resident-intruder tests, frequency of aggression was higher in AVP- than in either CSF- or OT-treated males ($F(2,22) = 4.98$, $P = 0.02$). (Fig. 1 legend; asterisk signifies $P < 0.05$ comparison with CSF). **b**, In a separate study, aggression (attacks + threats) increased above baseline within 12 h of beginning AVP infusion (1.0 ng h^{-1}). Asterisk signifies significant ($P < 0.05$) difference from baseline (Pre) aggression. Administration of a V_{1a} antagonist (50 ng, i.c.v.) at the start of infusion delayed the AVP-induced aggression by 18 h, yielding a temporal pattern significantly different from controls that received CSF i.c.v. instead of V_{1a} antagonist i.c.v. at the start of AVP infusion ($F(5,60) = 3.06$, $P < 0.05$). **c**, In a third group, during central infusion of CSF, OT or AVP (0.5 ng h^{-1}), each male was housed with a non-receptive female 'partner' for 24 h. In a subsequent preference test, CSF- and OT-treated males spent equal time with a novel female (stranger) and the partner ($t = 0.51$, NS and 0.17 , NS for CSF and OT, respectively). AVP-infused males spent more time with the partner than the stranger ($t = 3.26$, $P = 0.01$), with a significant overall treatment difference in time with the partner ($F(2,22) = 3.3$, $P = 0.05$) attributed to a difference between AVP- and CSF-treated males ($t_0 = 2.38$, $P = 0.05$).

METHODS. AVP, OT or CSF was given to sexually naive males using a chronic i.c.v. infusion technique. Following a baseline resident-intruder test, voles were anaesthetized with chloropent (chloral hydrate (40 mg ml^{-1}) plus pentobarbital (10 mg ml^{-1})) ($0.3 \text{ ml } 100 \text{ g}^{-1}$) and stereotactically fitted with a 25-G L-shaped cannula into the left lateral ventricle. The extra-cranial end of the cannula was fixed to the skull with dental cement and connected to PE20 tubing attached to an osmotic mini-pump (Alzet). The pump with attached tubing had been filled with CSF, OT or AVP (peptides from Peninsula) 18 h previously and primed by incubation in sterile saline at 37°C . After recovery from anaesthesia, males were returned to their home cages and tested for aggression towards an intruder at various times thereafter. In an initial study (a), AVP ($n = 9$), OT ($n = 8$) or CSF ($n = 8$) was administered via mini-pump and aggression was assessed 24 h later. In a second study (b), either CSF ($n = 6$) or $\text{d(CH}_2)_5[\text{ Tyr(Me)AVP}]$ (50 ng, $n = 6$) was administered through the cannula at the time of implantation to determine the time course and specificity of AVP effects. In a third study (c), males ($n = 25$) were implanted with pumps, housed with females for 24 h and then assessed for partner preference as described for Fig. 2.

has been reported to be within minutes¹⁵, a single injection of agonist may be insufficient to mimic physiological changes in AVP. To achieve prolonged elevated brain concentrations of peptide, we administered AVP (and OT) i.c.v. by osmotic mini-pump for 48 h to voles receiving neither sexual nor social experience. Males became aggressive after AVP (0.5 ng h^{-1}) but not after OT (0.5 ng h^{-1}) or CSF infusion (Fig. 3a). This dose of AVP increased AVP concentrations in the septum threefold ($0.94 \pm 0.29 \text{ pg per mg tissue}$ versus $0.31 \pm 0.10 \text{ pg per mg tissue}$ comparing AVP and CSF group; $n = 9$) using a standard radio-immunoassay (kit from Peninsula) with microdissected tissue after a 24-h infusion. Aggression appeared within 12 h of starting AVP administration and seemed to be mediated via a V_{1a} receptor, as prior injection with the V_{1a} antagonist delayed the initiation of aggression by 18 h (Fig. 3b). The aggressive behaviour after AVP seemed to be qualitatively similar to that seen post-mating, although the intensity of attacks was slightly less with exogenous peptide, possibly due to the encumbrance of the mini-pump. Nevertheless, the data demonstrate that activation of the V_{1a} receptor is both necessary and sufficient for the development of selective aggression in the male prairie vole.

To determine if exogenous AVP also induced a partner preference, a separate group of males received i.c.v. infusion of AVP, OT or CSF by mini-pump while being housed with an ovariectomized (that is, non-receptive) female for 24 h. In a 3-h choice test, CSF- and OT-injected males showed no preference (spent roughly equal times in partner's cage and that of the novel female). Males injected with AVP (0.5 ng h^{-1}) spent 75.4% of the available time with the partner and 16.7% with the stranger (Fig. 3c). The groups did not differ on number of entries into the two cages ($F(2,22) = 0.40$, not significant (NS)), suggesting that activity level was not affected by AVP.

Our results, implicating AVP in the development of selective aggression and partner preference, are consistent with recent data suggesting an important role for AVP in mediating parental behaviour in prairie voles⁸. Thus, it seems that AVP may be integral to several of the behaviours characterizing monogamous social organization in general and the process of pair bonding in particular. Studies in non-monogamous mammals have implicated AVP in social memory in rats¹⁰, territorial marking in hamsters⁹ and aggression in mice^{16,17}. There are also extensive data demonstrating AVP effects on memory and learning, thermoregulation, and grooming—effects with only an indirect relationship to affiliative behaviours, particularly in commonly used research animals such as rats and mice which do not pair bond (see, for instance, ref. 18). In a species that forms long-term selective bonds, however, memory of a mate, huddling for thermal comfort and grooming are important aspects of affiliation.

Our results are consistent with the hypothesis that the activation of central AVP pathways by mating is an essential neural correlate of pair bonding in the male prairie vole. Oxytocin and not AVP increases in CSF after ejaculation in the rat, a polygynous rodent¹⁹, whereas in male prairie voles AVP is apparently released after mating²⁰. AVP secretion has previously been described with sexual arousal in human males²¹, but the extent to which any single peptide subserves any aspect of social bonding in humans remains entirely speculative. □

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African and North American populations of *Drosophila melanogaster* are very different at the DNA level

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UNDERSTANDING genetic evolution within species requires an accurate description of variation within and between populations and the ability to distinguish between the potential causes of an observed distribution of variation. In the cosmopolitan species *Drosophila melanogaster*, previous studies suggested that gene flow within and between continents is extensive¹ and that most of the nuclear gene variation is found within, rather than among, populations^{2,3}. Here we present evidence that a population from Zimbabwe is more than twice as variable as those from the United States of America at the DNA sequence level, that most variants are not shared between the two geographic regions, and that there are nearly fixed differences between the Zimbabwe and USA samples in genomic regions experiencing low recombination rates. It appears that there is an unappreciated degree of population structure in *D. melanogaster* and that equilibrium models of molecular evolution are inappropriate for this species.

In Table 1 we present our four-cutter restriction site polymorphism data from seven X-linked gene regions in *D. melanogaster* chromosomes sampled from Zimbabwe; we also show our data and previously published data from the same genes in samples from the USA^{4–7}. With the exception of *ac*, each gene is more variable in Zimbabwe than in the USA. Overall, there are more than twice as many segregating sites in Zimbabwe (167) as in the USA (77). The difference in proportion of segregating sites in the two samples is highly significant (Fisher's exact test, $P < 0.0001$; this test is appropriate because the sample sizes for Zimbabwe and the USA are roughly equal) and the populations are significantly differentiated ($P < 0.001$) at each of the seven genes examined⁸. Only 44 of a total of 200 polymorphic restriction sites (22%) scorable in both Zimbabwe and the USA are observed in both geographic regions. This difference is not attributable solely to rare 'private' alleles. For example, in the USA the mean frequencies of *white* and *G-6pd* locus variants present in the USA but absent from Zimbabwe are 0.16 (± 0.042) and 0.23 (± 0.058), respectively. The contrasting Tajima *D* values⁹ (Table 1) for the two samples (especially in regions of 'normal' crossing-over) indicate that there is a tendency for Zimbabwe to harbour more low-frequency variants than the USA, providing further evidence of different evolutionary processes in these populations. But the difference in variability is not simply a consequence of the presence of rare alleles in Zimbabwe which are absent in the USA. Even for estimates of nucleotide diversity, π , which are only weakly affected by rare variants, the Zimbabwe sample is two- to sixfold more heterozygous for most loci.

Our results contrast with previous comparisons of nuclear gene variability in African and USA samples of *D. melanogaster*.

Restriction site data suggested that the *y-ac-sc* and *G-6pd* loci were slightly more variable in Botswana than in the USA; however, the high levels of linkage disequilibrium and small number of bases surveyed made it difficult to assess the significance of the observation^{10,11}. Levels of allozyme variation in Benin and USA samples of *D. melanogaster* (Table 2) were virtually identical and 57 of 84 allozyme variants (68%) were shared between geographic regions^{2,12}. The difference in the proportion of shared variants in our restriction site data versus the allozyme data is highly significant (Fisher's exact test, $P < 0.0001$; this test is conservative given the different sample sizes for the USA and Africa allozyme data). Thus, our four-cutter data are the first good evidence that there is a vast reservoir of previously unknown nuclear DNA polymorphism segregating in Africa.

Although it is thought that *D. melanogaster* left Africa to colonize temperate regions within the past several thousand years¹³, earlier data did not support the hypothesis that a wholesale loss of nuclear gene variability accompanied the colonization. Our data, however, are consistent with the notion that a considerable loss of variation occurred in some populations during the history of the *D. melanogaster* lineage. The data are compatible with several hypotheses, including a partial bottleneck in the lineage(s) from which USA populations are derived and the notion that only a subset of 'ancestral' types were selectively favoured to leave Africa during the colonization process. Whatever the case, it is no longer realistic to view *D. melanogaster* populations as being near equilibrium.

Earlier interpretations of allozyme data from *D. melanogaster* attributed the few frequency differences between populations to selection on allozyme variants prevailing over extensive gene flow^{1,2}. Because a large majority of observed polymorphic restriction sites in our data are located in flanking or intronic sequences, most of the differences between Zimbabwe and the USA may be explained in terms of mutation and drift rather than selection. Furthermore, our results cannot be explained solely by differences in the frequency of protein electrophoretic variants between populations. For example, even within a single electromorph class at *Pgd* (Fast), the Zimbabwe (sample size $n = 49$) and USA ($n = 32$) samples shared no four-cutter haplotypes and only 7 of 28 polymorphic sites (data not shown). There are at least two (not mutually exclusive) potential explanations for the contrast between the DNA data (Table 1) and the allozyme data (Table 2). First, there may be significant population structure within Africa; we note that our Zimbabwe sample has not been surveyed for allozyme variation. Second, we cannot rule out the possibility that restriction site and allozyme variation are experiencing substantially different evolutionary dynamics (such as selection on allozyme variation).

The Zimbabwe four-cutter data provide novel and qualitatively different inferences about selection from those possible from allozyme frequencies. As had been seen in a heterogeneous sample¹⁴, nucleotide heterozygosity is positively correlated with regional rates of recombination (Fig. 1). Hitch-hiking effects of advantageous or deleterious alleles have been proposed to contribute to this pattern^{14–17}. In particular, the *ac* and *su(f)* regions appear to have significantly less variation than expected under a neutral model (Table 2 legend). Although several shared polymorphic sites in Zimbabwe and the USA are found at different frequencies in the two samples, the most extreme cases

TABLE 1 Four-cutter restriction site data from *D. melanogaster* samples collected in Zimbabwe and the USA

Gene	Sites scored		Polymorphic sites			$\hat{\theta}(3N\mu)$		$\hat{\pi}$		Tajima <i>D</i>		F_{ST}	cM per band
	Zim.	USA	Zim.	USA	shared	Zim.	USA	Zim.	USA	Zim.	USA		
<i>y</i>	102	80	8	2	0	0.0026	0.0008	0.0017	0.0009	-0.433	0.469	0.56	<0.003
<i>ac</i>	68	62	2	2	1	0.0010	0.0011	0.0012	0.0011	0.789	0.260	0.54	<0.003
<i>su(f)</i>	171	108	6	2	0	0.0011	0.0005	0.0011	0.0002	0.338	-0.842	0.60	<0.003
<i>Pgd</i>	200	200	25	14	9	0.0042	0.0021	0.0018	0.0024	-1.497	0.922	0.25	0.010
<i>G-6pd</i>	226	175	35 (48)	16 (17)	9	0.0076	0.0030	0.0048	0.0025	-0.888	-0.009	0.30	0.033
<i>vermillion</i>	80	76	25	9	9	0.0118	0.0045	0.0082	0.0042	-0.804	-0.273	0.32	0.067
<i>white</i>	285	271	66 (73)	32 (33)	16	0.0094	0.004	0.0065	0.004	-0.714	0.136	0.28	0.093

Loci are listed in order of increasing recombination (centimorgans (cM) per band). Homozygous X-chromosome lines were made with the *FM7a* balancer. The Zimbabwe sample ($n = 50$) was collected at the Sengwa Wildlife Preserve in September 1990. The USA *Pgd* ($n = 53$) and *vermillion* ($n = 35$) samples were from North Carolina and California, respectively. Ten four-cutter restriction enzymes were used (*AluI*, *DdeI*, *HaeIII*, *HhaI*, *HinfI*, *MspI*, *RsaI*, *Sau3AI*, *ScrFI* and *TaqI*) to make blots as previously described²⁰. USA data from *y*, *ac*, *su(f)*, *G-6pd* and *white* are from a set of X-chromosomes collected from North Carolina ($n = 20$) and Texas ($n = 27$) as previously described^{4,7}. For *y*, *ac*, *su(f)*, *G-6pd* and *white* the set of enzymes used differed slightly from the Zimbabwe sample. For the column 'Polymorphic sites', numbers not in parentheses show the sites that could be scored in both USA and Zimbabwe given the enzymes used and the genomic regions surveyed; numbers in parentheses show the total number of polymorphic sites observed. Calculation of θ , π , D and F_{ST} were as described^{9,21,23}. $\hat{\theta}$ is an estimate of nucleotide variability based on the proportion of polymorphic nucleotides. Assuming a neutral equilibrium model, $\hat{\theta}$ equals the parameter $3N\mu$ (for X-linked genes) where N and μ are the effective population size and the neutral mutation rate, respectively. $\hat{\pi}$ is an estimate of nucleotide heterozygosity or the average pairwise difference per nucleotide between two randomly selected sequences. Tajima's D provides an estimate of the magnitude of the difference between the estimates of nucleotide variation provided by $\hat{\theta}$ and $\hat{\pi}$ and is expected to equal zero under a neutral equilibrium model. Tajima's D becomes more negative as the number of rare mutations increases and becomes more positive as the number of intermediate frequency mutations increases. F_{ST} provides an estimate of population differentiation based on the proportion of total heterozygosity found within versus between populations. We used the HKA test²⁴ to assess the hypothesis that Zimbabwe *ac* and *su(f)* polymorphism within *D. melanogaster* and divergence to *D. simulans*^{6,7} compared to a sample of 5' *Adh*²⁵ are consistent with a strictly neutral model. The HKA test is significant for both *ac* ($\chi^2 = 4.05$, $P < 0.05$) and *su(f)* ($\chi^2 = 12.83$, $P < 0.001$); the best interpretation is that there is less variation at *ac* and *su(f)* than expected under a neutral model of molecular evolution. This is a conservative test for reduced polymorphism at *ac* and *su(f)* because the Zimbabwe population appears to be considerably more variable than Kreitman's sample. The *ac* and *su(f)* gene regions also showed a significant reduction in variability in the USA^{6,7}. cM per band was estimated by dividing the genetic distance between markers which flank the gene of interest by the number of polytene bands as determined from Bridge's maps²⁶. cM per band are multiplied by 0.666 to correct for the absence of recombination in males¹⁴. The value for cM per band for *vermillion* is greater than in our previous report¹⁴. This discrepancy results from a more detailed analysis of the recombination rate in the region which revealed that *vermillion* is in a region where recombination appears to increase rapidly. We believe the value presented in this report is more accurate. cM per band for *y*, *ac* and *su(f)* are uncertain because of poor genetic and/or physical data and are probably overestimates.

of differentiation are found in regions of reduced recombination (Tables 1 and 3). The joint observations of genomic regions with significantly reduced variation within populations and nearly fixed differences between populations are best explained by independent hitch-hiking events¹⁸. Whether the selective histories of Zimbabwe and USA samples differ because of isolation, as opposed to varied environments, is an important, unanswered question. A potential problem for a simple hitch-hiking interpretation is the lack of skewness toward rare variants in regions of reduced crossing-over predicted by some models¹⁹; the 16 polymorphisms observed in *y*, *ac* and *su(f)* from Zimbabwe have less skewness than the polymorphisms located in regions of 'normal' crossing-over. Further theoretical research is necessary to explain contrasting patterns of variation in genomic regions experiencing different recombination rates.

Our data provide convincing evidence that *D. melanogaster*,

TABLE 2 Allozyme data from USA and African samples of *D. melanogaster*

	Massachusetts (USA) (n = 30)	Texas (USA) (n = 30)	Benin (West Africa) (n = 28)
Proportion polymorphic loci	0.43 (0.81)	0.36 (0.81)	0.39 (0.77)
Average number of alleles	1.65 (2.48)	1.55 (2.52)	1.66 (2.64)
Heterozygosity	0.13 (0.28)	0.12 (0.31)	0.11 (0.27)

Summary statistics from previously published allozyme surveys^{2,12}. Numbers not in parentheses are from a random sample of 117 loci, and numbers in parentheses are from a sample of 26 polymorphic loci.

TABLE 3 Restriction sites in regions of reduced recombination showing large frequency differences between Zimbabwe and the USA

Gene	Site	Site frequency (+)	
		Zimbabwe	USA
<i>y</i>	<i>HaeIII</i> 4444	0.00	0.79
<i>ac</i>	<i>TaqI</i> 36	1.00	0.15
<i>su(f)</i>	<i>DdeI</i> 2242	0.00	0.91

USA data and site designations are as previously described^{6,7}.

as a species, is far more variable than previously thought, and that most of this variation is not segregating in the populations from which our evolutionary inferences (effective population size, frequency of lethal mutations, transposable element copy number and so on) and models are derived. It is no longer tenable to think of *D. melanogaster* as essentially panmictic for nuclear genes. Furthermore, it is no longer realistic to assume that *D. melanogaster* populations are near equilibrium or that conclusions derived from studies of USA (or similar) samples are even roughly true for the species as a whole. □

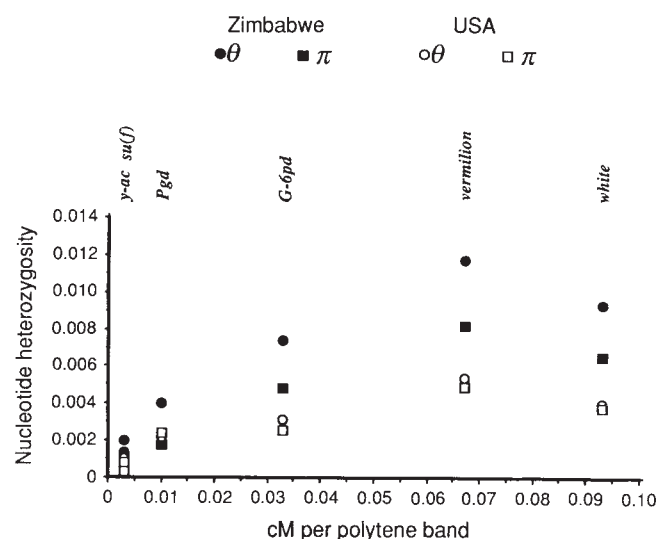


FIG. 1 Scatterplot of nucleotide heterozygosity in Zimbabwe and USA *D. melanogaster* versus cM per polytene band. Data are from Table 1.

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Macaque V1 neurons can signal 'illusory' contours

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We describe here a new view of primary visual cortex (V1) based on measurements of neural responses in V1 to patterns called 'illusory contours' (Fig. 1*a, b*). Detection of an object's boundary contours is a fundamental visual task. Boundary contours are defined by discontinuities not only in luminance and colour, but also in texture^{1,3}, disparity⁴ and motion^{5,7}. Two theoretical approaches can account for illusory contour perception. The cognitive approach emphasizes top-down processes^{8,9}. An alternative emphasizes bottom-up processing. This latter view is supported by (1) stimulus constraints for illusory contour perception^{10–14} and (2) the discovery by von der Heydt and Peterhans^{15–17} of neurons in extrastriate visual area V2 (but not in V1) of macaque monkeys that respond to illusory contours. Using stimuli different from those used previously^{15,16}, we found illusory contour responses in about half the neurons studied in V1 of macaque monkeys. Therefore, there are neurons as early as V1 with the computational power to detect illusory contours and to help distinguish figure from ground.

We measured responses of 25 neurons in para-foveal V1 of anaesthetized monkeys to four types of patterns sharing the same mean luminance: sinusoidal luminance gratings (Fig. 1*c*); luminance edges (Fig. 1*d*); half-screen patches of sinusoidal luminance grating, in which the bar ends define an illusory contour oriented perpendicularly to the bars and located at the boundary between the grating patch and a blank portion of the stimulus screen at the same mean luminance (Fig. 1*e*); and two half-screen patches of grating abutting one another, in which the two patches are identical and in antiphase (Fig. 1*f*). Results obtained using the pattern in Fig. 1*f* resemble those shown in Fig. 1*e* (for example, the cell in Fig. 2*a* responded similarly to both).

Because the grating is periodic, it is natural to express its position relative to receptive field location as spatial phase: the starting spatial phase angle of the sinusoidal grating relative to the stimulus frame. For example, in Fig. 1*e* the spatial phase is 0 degrees. As phase varies, the position of grating bars with respect to fixed cell receptive fields varies so that, when the illusory contour is drifted, different values of black and white are swept across a receptive field.

We found responses to illusory contours drifted across a cell's receptive field in the preferred direction for a luminance edge (that is, when grating bars defining the illusory contours were perpendicular to the optimal orientation for an edge). A variety of responses to illusory contour patterns was observed. One type

is illustrated in Fig. 2*a*: this direction-selective complex cell fired two bursts per period, one to each of the 'left-handed' and 'right-handed' boundaries of the central patch in Fig. 1*e, f*. Comparison of responses to luminance (Fig. 1*d*) and illusory contours (Fig. 1*e, f*) showed that the two bursts were synchronized to the passage of the illusory contour over the cell's receptive field. The cell in Fig. 2*a* signals the presence of a contour in its receptive field independent of contrast polarity: the stimulus when the illusory contour leaves the receptive field is opposite in sign to when it enters, but the cell's response is excitatory to both entry and exit. Our measure of this frequency-doubled response is the Fourier amplitude of the second harmonic (F2) of the post-stimulus time histogram. Another type of cell responded to just one of the two contours passing over the receptive field, illustrated by the response shown in Fig. 2*b*.

If a frequency-doubled response signifies the presence of illusory contours crossing a cell's receptive field, its magnitude should not depend on the precise position (spatial phase) of the illusory contour along the long axis of the receptive field. Figure 2 shows how response changed for two cells as spatial phase varied. The simple cell in Fig. 2*b* responded to the leading edge of the one-sided pattern from 1.75 π to 0.25 π radians, and to the trailing edge from 0.75 π to 1.25 π radians. This implies that the cell was responding simply to a bright bar-end entering the receptive field or to a dark bar-end leaving it. But the response of the cell shown in Fig. 2*a* was the same at all spatial phases, implying that it responded to contour and not simply to bar-ends.

An index of response-dependence on spatial phase was derived: the greatest (pairwise) difference in response, divided by the sum of the maximum and minimum response. Figure 3 shows the distribution of the index across complex cells (*a*) and simple cells (*b*). For most complex cells this index was less than 0.5; the indices of simple cells were distributed uniformly between 0–1. This implies that many cortical cells responded to extended contour, rather than to individual grating bar endings. Of the nine cells with phase modulation index of less than 50%, the median relative contrast sensitivity (grating threshold/illusory contour threshold) was 0.76 (range, 0.32–4.0). This means that the illusory contours were effective stimuli. We did not measure the length of the neurons' receptive fields, but spatial phase invariance implies nonlinear spatial summation along the grating-defined contour. A modification of the Spitzer-Hochstein¹⁸ model for cortical complex cells may account for the illusory contour responses we observed. Misaligning that model's subunits in the axis parallel to the cell's preferred orientation, so that each subunit has a different centroid of sensitivity in the 'long axis' of the receptive field, will yield spatial phase insensitivity.

Responses to illusory contours defined by line gratings (as in Fig. 1*b*) were compared with those defined by sine gratings in eight neurons. The linewidth used was 1.2 arcmin, chosen to be like line-grating stimuli used in refs 15–17. The median ratio of peak responses to line/sine-defined illusory contours was 0.2. The linewidth in the line-defined illusory contour seems to be a

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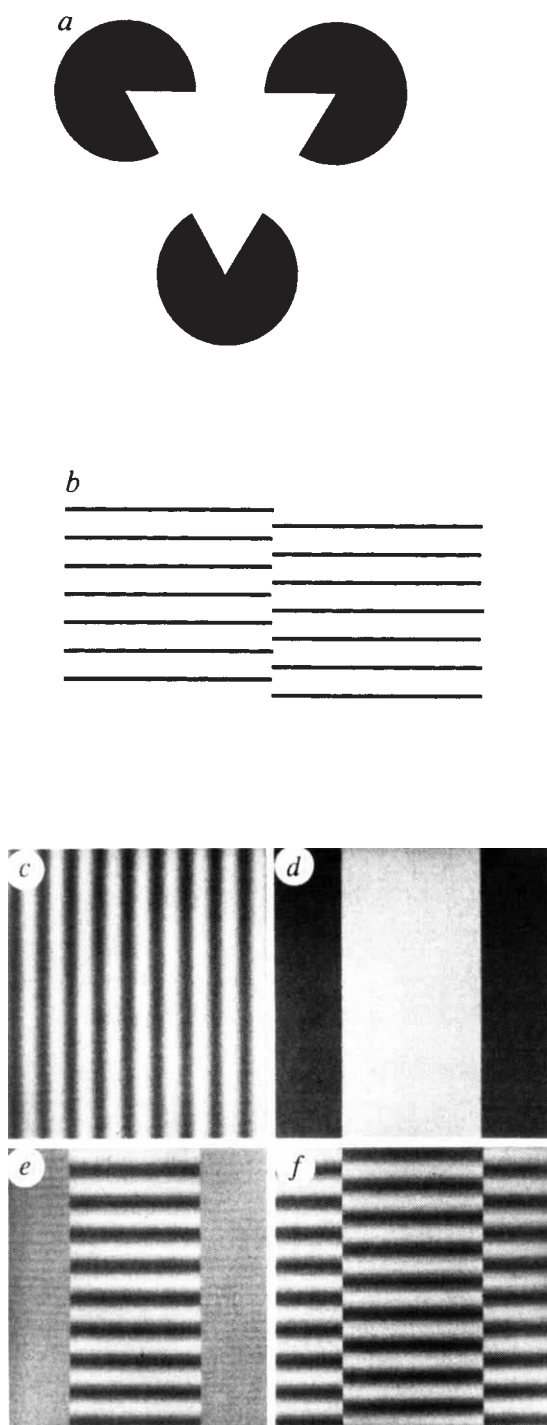


FIG. 1 Visual patterns. *a*, Kanizsa triangle. A triangle appears to occlude three circular disks. There is no change in luminance across the middle of each side of the triangle. *b*, Abutting line grating. A sharp edge appears where the two line gratings meet. *c*, Full-field sinusoidal grating. *d*, Simple luminance edges. Two edges of opposite contrast polarity divide the display into two rectangular patches. Motion direction is normal to the edges in *a* and *b*. *e*, 'One-sided' illusory contour pattern. A half-screen patch of grating defines two illusory contours, one on each side of the patch. The contours are perpendicular to the grating bars. The mean luminance of the blank portion of the screen is the same as that of the grating patch. *f*, 'Two-sided' illusory contour pattern. Two gratings differing only in their relative vertical position (spatial phase) define illusory contours at both abutments. The pattern motion was rigid horizontal translation in *c* and *d*, and normal to the vertical illusory contours in *e* and *f*.

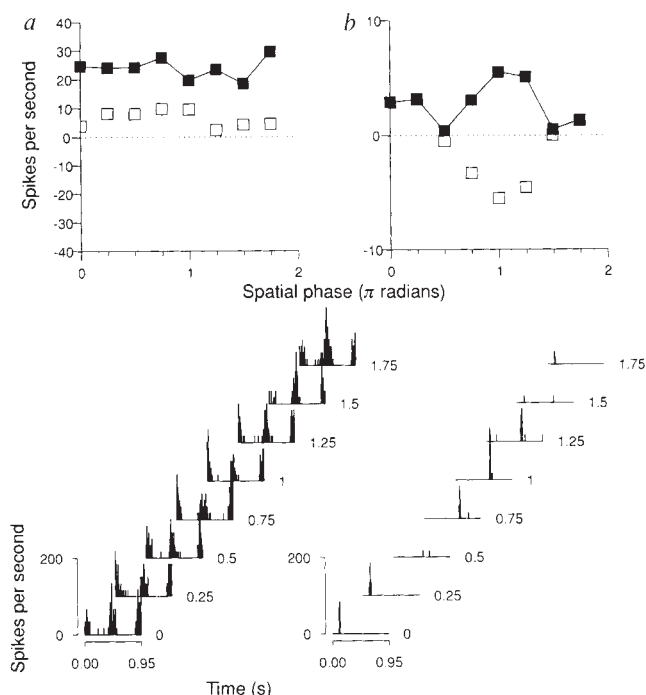


FIG. 2 Two types of striate response to illusory contour patterns of Fig. 1e. *a*, The lower series shows the peristimulus time histograms (PSTHs) of a layer-5, directionally selective, complex cell's responses to a one-sided (as in Fig. 1e) illusory contour pattern (1.3 cycles per degree grating, 80% contrast), drifted at 6 deg s^{-1} in the optimal edge direction and with the spatial phase (in π radians) written alongside each PSTH. Each pattern drifted to the edge of the screen, wrapped around, and returned to its starting position every 947 ms. Histogramming the spikes using a temporal period of 947 ms shows the cell responded to each of the two illusory contours passing over the receptive field in a period. The upper plot shows the amplitude of the first (F1, open squares) and second (F2, filled squares) harmonics of the PSTHs. The phase modulation index (see text) for this cell was 0.27. *b*, The lower plots show PSTHs of a simple cell in layer 5 to one-sided illusory contour patterns (0.7 cycles per degree grating, 80% contrast). The pattern is drifted at 6 deg s^{-1} in the optimal direction, at a series of spatial phases denoted to the right of each PSTH. As spatial phase is varied, the cell responds to one or the other of the illusory contours but never to both. The response virtually nulls at two spatial phases, spaced a half-cycle apart. The upper plot shows F1 and F2 varying with spatial phase. The sign of the F1 response is made negative to denote when temporal phase of the F1 harmonic changes sign. The near-sinusoidal dependence of F1 response on spatial phase is a signature of mechanisms with spatial summation linearity²³. The phase modulation index is 0.80. METHODS. Standard procedures were used in these acute recording experiments of cynomolgus monkeys (*Macaca fascicularis*; 2–6 kg). Anaesthesia was induced with ketamine (12–16 mg per kg body weight) intramuscularly and maintained with sufentanyl ($6 \mu\text{g kg}^{-1} \text{ h}^{-1}$, intravenous). Local anaesthetic was applied to pressure points and incisions; electroencephalogram, electrocardiogram and blood pressure were monitored and anaesthesia supplemented as necessary with either acepromazine or sufentanyl. Extracellular action potentials were recorded from single neurons using glass-coated tungsten microelectrodes²⁴ with 5–15- μm exposed tips. Quantitative measurement was done with patterns displayed to the preferred eye on either a Tektronix 608 (P4 phosphor, 10 cm by 10 cm, 270-Hz frame rate, mean luminance 100 cd m^{-2}) CRT monitor or a colour Tektronix 690 (25.6 cm by 25.6 cm, 135 Hz frame rate, 67 cd m^{-2}) monitor. The scopes were controlled and gamma-corrected by a custom graphics instrument²⁵ and a PDP 11–83 computer. The cathode ray tubes were also calibrated with a Photo Research Model 703-PC spectroradiometer to make certain that there was no artefactual luminance difference between the blank and textured regions of the stimulus. Cells were classified using a modification²³ of the Enroth-Cugell and Robson null test. Histological processing and laminar assignment of neurons were as described in refs 26, 27.

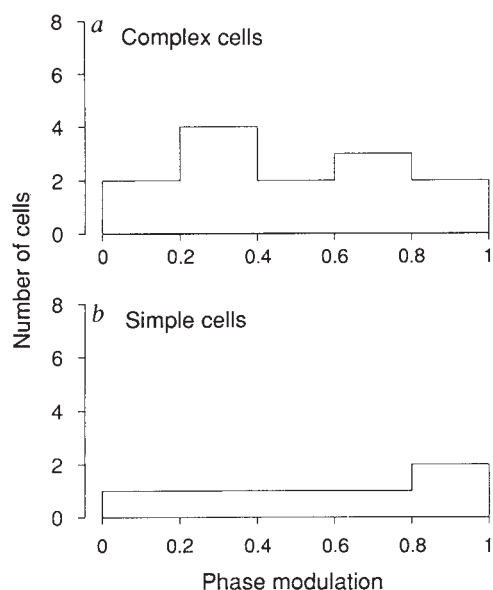


FIG. 3 Phase modulation index for: a, 13 complex and b, 6 simple cells. The index is the Michelson contrast of the array of F2s evoked by stimuli at different spatial phases: greatest (pairwise) difference in response divided by the sum of the maximum and minimum response. The index indicates whether a response to the illusory contour depends on the precise position of the grating patch perpendicular to the illusory contour: an index of one means the cell has a null spatial phase and zero means the response is invariant to changes in spatial phase. The stimulus contrast of the gratings used to induce the contours was 60–80%.

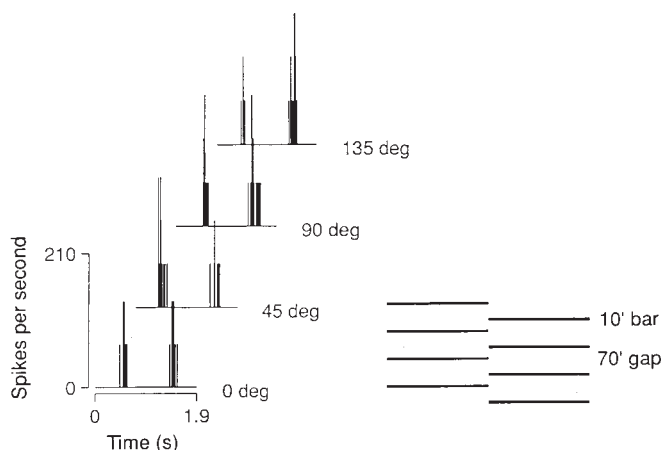


FIG. 4 Response of a V1 complex cell to abutting line gratings. The stimulus is depicted on the right side of the figure; each grating had a period of 80 arcmin, with a linewidth of 10 arcmin, and a gap between lines of 70 min. The gratings were always offset from each other by half a cycle, irrespective of spatial phase, creating an illusory contour at the line of abutment, as in Fig. 1b. The bars' luminance was $\sim 0.5 \text{ cd m}^{-2}$ against a background of 100 cd m^{-2} . The left side of the figure depicts the responses of the neuron at four different spatial phases, with the same format as in Fig. 2. The pattern was drifted continuously, with 'wraparound' and the drift speed was 2.3 deg s^{-1} ($\sim 0.5 \text{ Hz}$).

critical variable. We were able to measure phase-insensitive illusory contour responses in a V1 neuron with line gratings in which the lines were 10 arcmin wide (Fig. 4); however, this same neuron did not respond to line gratings with lines 1 arcmin wide.

The ability of illusory contour-responsive neurons to ignore contrast polarity and boundary type suggests that they convey information about the location and orientation of object borders salient for segmentation^{19, 21}. These results indicate that V1 neu-

rons perform sophisticated image processing and that V1 is an image-processing area, rather than simply a spatially transformed replication of the retina. Our results and conclusions are consistent with a recent report²² that motion-defined contours also produce responses in V1 cortex. □

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Protein tyrosine kinase p56^{lck} controls allelic exclusion of T-cell receptor β -chain genes

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DURING T-cell development, site-specific DNA rearrangements mediating assembly of β - and α -chain genes of the T-cell receptor (TCR) are developmentally ordered^{1,2}. In particular, assembly and expression of a complete β -chain gene blocks further rearrangements at the β -locus (a process referred to as allelic exclusion)³ and drives the generation and expansion of CD4⁺8⁺ cells^{4,5}. Although the mechanism used by TCR β chains to deliver such signals is unknown, studies in transgenic animals have suggested that the lymphocyte-specific protein tyrosine kinase p56^{lck} may impinge on a similar signalling pathway⁶. The hypothesis that TCR β chains deliver intracellular signals via p56^{lck} makes an explicit prediction: that interference with p56^{lck} function will mitigate the effects of a simultaneously expressed TCR β chain. Here we confirm this prediction through examination of allelic exclusion in mice expressing both a functional TCR β chain transgene and a catalytically inactive form of p56^{lck}.

p56^{lck} participates in TCR-derived signalling⁷ in part through a physical association with the coreceptors CD4 and CD8^{8,9}. In addition, a critical role for this kinase during early thymocyte

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development (before CD4 or CD8 expression commences) has been identified using *lck^{null}* mice¹⁰. A detailed analysis of the importance of p56^{lck} in regulating thymocyte development was recently achieved through the generation of a dominant-negative *lck* transgene containing an arginine for lysine substitution at residue 273¹¹. Expression of catalytically inactive p56^{lckR273} yields results analogous to those seen in *lck^{null}* mice: a transgene dose-dependent decrease in thymocyte number corresponding to a blockade in the generation of CD4⁺8⁺ cells.

Flow cytometric profiles of CD4 and CD8 expression on thymocytes from control and from three independent lines of *lckR273* animals are shown in Fig. 1a. Although thymocytes from the highest expressing line, 11924, contain normal levels of TCR V β rearrangements¹¹, the thymic phenotype in these animals is similar to that seen in mice that fail to express TCR β chains, such as RAG-1¹², RAG-2¹³ or TCR β + δ ⁴ mutant mice. For each of the latter recessive mutants, introduction of a transgene construct directing expression of a functional TCR β chain can rescue both the number and proportion of CD4⁺8⁺ cells^{4,5}. Hence a TCR β chain-derived signal promotes thymocyte maturation. In contrast, a β -chain transgene does not correct the developmental defects in *lckR273* animals (Fig. 1a). Both the reduced thymocyte number and the disproportionate representation of immature CD4⁺8⁻ cells remain unchanged. Most CD4⁺CD8⁻ cells in both single- and double-transgenic thymi had the size characteristics of thymoblasts and expressed the IL-2R α chain (data not shown). Thus the simple presence of a functional β -chain gene cannot by itself overcome the dominant defective phenotype conferred by the presence of the *lckR273* transgene.

Failure of the transgenic β -chain to drive differentiation in *lckR273* mice is not due to a lack of expression, because flow cytometric profiles (V β 8 versus CD3) indicate that over 95% of double-transgenic thymocytes display surface V β 8 molecules (Fig. 1b). In two independent lines of *lckR273* mice (13413 and 11711), and in control animals, this results in a linear relationship between CD3 and V β 8 surface expression on thymocytes such that both CD3^{lo}/V β 8^{lo} and CD3^{hi}/V β 8^{hi} cells can be visualized. The 11924/ β TG⁺ thymuses, however, contain very few thymocytes and all of these exhibit a homogeneous CD4⁺8⁻ phenotype (described in detail for the 11924 *lckR273* mice in ref. 11). This homogeneous character permits only a single intermediate level of both CD3 and V β 8 expression in 11924/ β TG⁺ thymocytes. Despite uniform expression of the V β 8 transgene product, the 11924/ β TG⁺ thymuses, like those seen in single-transgenic 11924 animals, contained less than 10% the normal number of thymocytes, and almost no CD4⁺8⁺ cells (Fig. 1a). These observations are consistent with the view that p56^{lckR273} blocks delivery of a TCR β chain-derived signal that ordinarily promotes thymocyte development. Alternatively, the *lckR273* transgene could exert a preemptory, biochemically unrelated, signal that by chance yields a phenotype identical to that seen in β ^{null} mice.

A better assessment of the importance of p56^{lck} in TCR β chain signalling was achieved through analysis of TCR gene rearrangements. Expression of a functional β -chain transgene in thymocytes ordinarily blocks endogenous β -locus gene rearrangements almost completely³. The fidelity of this 'allelic exclusion' process could therefore be examined in *lckR273*/ β TG thymocytes using a polymerase chain reaction (PCR) assay which provides an accurate representation of the relative levels of gene rearrangements in various samples⁶. Primers specific for individual V β elements in combination with a primer positioned 3' to the J β 2 cluster yield PCR products corresponding to rearrangements involving each of the six J β 2 elements. As shown previously⁶, β -chain single-transgenic thymocytes (Fig. 2, lanes 3 and 7) contain nearly undetectable levels of endogenous V β rearrangements. But the simultaneous presence of the *lckR273* transgene mitigates the effects of the transgenic β -chain, permitting rearrangements of endogenous TCR V β gene segments to occur at very substantial levels (compare Fig. 2 lanes 2, 6 and 10 with

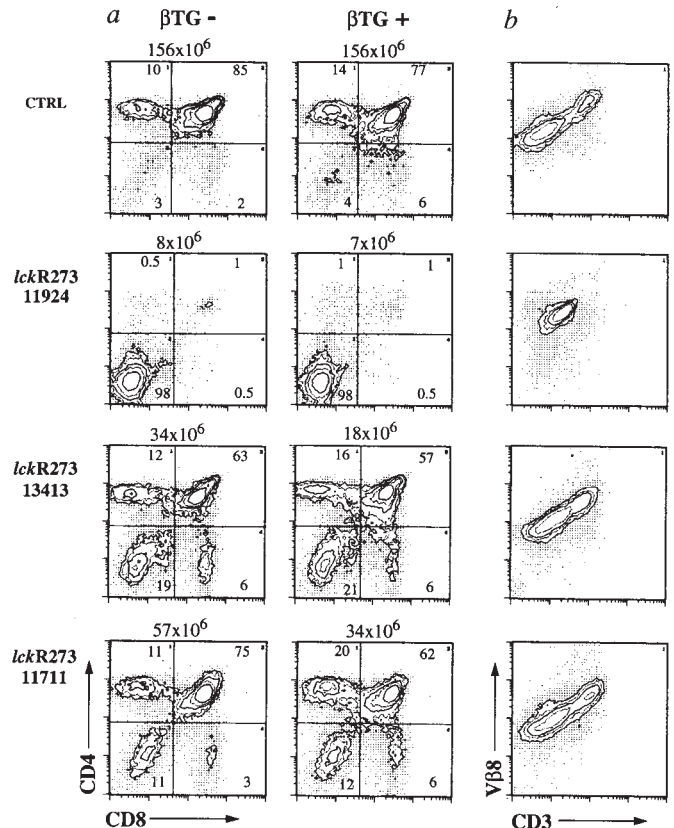


FIG. 1 Flow cytometric analysis of thymocytes from *lckR273* and TCR β -chain transgenic mice. a, Fluorescence staining profiles for surface CD4 and CD8 expression on β -chain transgene negative (left) or positive (right) thymocytes. The three *lckR273* parental lines which were crossed with β -transgenic mice are given numeric designations at the left of each set of histograms. Profiles from littermate β TG⁻ and β TG⁺ animals for each line (except 11924 mice, which were simply age-matched), are shown. Total thymocyte number is listed above each plot, and the percentage of cells in each quadrant is indicated. b, Profiles for surface V β 8 and CD3 expression on β TG⁺ thymocytes from the same animals shown in a.

METHODS. Breeding pairs of *lckR273*¹¹ and β TG³ animals were established, and offspring were typed by PCR using primers specific for each transgene. Thymocytes were isolated from littermates 3 to 4 weeks old and stained for flow cytometric analysis as described⁶ with the following monoclonal antibodies: phycoerythrin-conjugated GK1.5 (anti-CD4, Becton Dickinson); fluorescein-conjugated 53.6.71 (anti-CD8, Becton Dickinson); biotin-conjugated F23.1 (anti-V β 8, a gift from P. Fink) detected with phycoerythrin-conjugated streptavidin (Caltag Labs); and fluorescein-conjugated 2C11 (anti-CD3 ϵ). Each analysis included 2×10^4 events collected in list mode on a FACScan flow cytometer (Becton Dickinson) and analysed using FACstar Consort 30 software.

control lanes 1, 4 and 8). Although not precisely quantitative, the PCR results indicate that the highest levels of V β rearrangements are found in the line expressing the highest level of *lckR273*. Thus expression of the dominant-negative *lck* transgene results in an unprecedented violation of allelic exclusion at the β -chain locus, in accord with the hypothesis that p56^{lck} mediates the TCR β chain-derived signal transduction process that controls thymocyte development.

PCR analysis also revealed that V α rearrangements did not occur in thymocytes from the TCR β transgene-bearing 11924 animal (lane 2), as was previously observed in singly transgenic *lckR273* animals from this line¹¹. This observation suggests that the ability to support rearrangements at the α locus may require p56^{lck}-derived signals. Although TCR α chain gene rearrange-

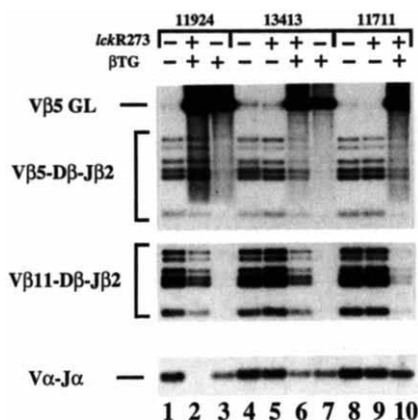


FIG. 2 Quantification of relative levels of V β and V α rearrangements in thymocyte DNA. Top, PCR products representing rearrangements of V β 5 or V β 11 to the six J β 2 elements (J β 2.1–J β 2.6) were visualized after hybridization with a 3'J β 2-specific oligonucleotide probe. In addition, a band corresponding to a germ line, unrearranged V β 5.1 element (V β 5 GL) positioned 5' to rearranged V β 8.2 gene segments can be amplified because of the tandem arrangement and proximity of the V β 5 and V β 8 germ-line elements²⁸. This configuration is present in the β -chain transgene³ and gives rise to the intense V β 5 GL band seen in β TG+ thymocytes. Bottom, Rearrangements of members of the V α 1, V α 3, V α 8 families to J α pHDS58.

METHODS. PCR amplification, gel electrophoresis, and detection by blot hybridization of V β and V α rearrangements from thymocyte DNA used conditions previously described⁶. Sequences of the V β 5, V β 11, J β 2, V α 1 (Va5H), V α 3 (Va2C), V α 8 (VaF3) and J α pHDS58 primers and probes are as reported^{6,11}.

ments do occur at reduced levels in TCR β^{null} mice⁴ and in mice bearing a targeted disruption of the *lck* locus¹⁰, it is possible that relatively little protein tyrosine kinase activity, perhaps that derived from a small population of basally stimulated kinase molecules, is sufficient to permit TCR α locus rearrangements. Consistent with this view, V α rearrangements were detected in animals expressing lower levels of the dominant-negative *lck* transgene (13413 and 11711, Fig. 2).

Reconstitution of RAG and TCR β mutant mice with TCR β chain transgenes has confirmed that expression of a functional β -chain stimulates the generation and expansion of CD4⁺8⁺ cells during thymocyte development^{4,5}. Evidence that p56^{lck} might relay these β -chain-derived signals came from two sources. First, overexpression of an activated form of p56^{lck} (bearing a phenylalanine for tyrosine substitution at position 505) blocks V β rearrangement in developing thymocytes without compromising the ability to generate normal numbers of CD4⁺8⁺ cells⁶. Hence p56^{lck} activity can promote thymocyte differentiation in the absence of a TCR β chain. Second, both targeted disruption of the *lck* gene¹⁰ and expression of a catalytically inactive p56^{lck}R273 transgene¹¹, prevent the normal generation of CD4⁺8⁺ thymocytes. Thus the phenotypes resulting from increased and decreased *lck* activity parallel those provoked by the presence or absence, respectively, of a functional TCR β chain (the actions of *lck* transgenes during early thymocyte development are presented schematically in Fig. 3). Based on these data, we hypothesized that p56^{lck}, which is expressed at highest levels in immature thymocytes^{14–16}, serves as the sensing mechanism for satisfactory formation of a functional TCR β chain gene⁶. Consistent with the predictions of this model, Fig. 2 demonstrates that interference with p56^{lck} function prevents allelic exclusion at the TCR β locus.

Although p56^{lck} is best known for its ability to interact with the CD4 and CD8 coreceptors^{8,9} and with the IL-2 receptor β -chain¹⁷, defects in *lck* expression may compromise signalling from the TCR itself^{18,19}. Hence there is reason to believe that a

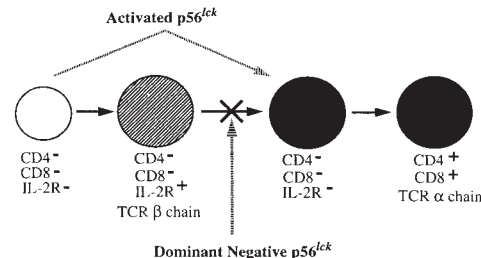


FIG. 3 Schematic diagram of the effects of *lck* transgenes on early thymocyte development. The dominant negative *lck*R273 transgene interferes with the TCR β chain-dependent transition from IL-2R⁺ thymoblasts (hatched circles) to the more mature, replicating CD4⁺CD8⁺ and CD4⁺CD8⁺ stages (filled circles). The pattern of expression of the IL-2R α chain at each stage is also noted (see ref. 11 for more details).

TCR-derived signal in immature thymocytes might act via p56^{lck}. The ability of the TCR β chain to associate with CD3 signalling components of the TCR in the absence of TCR α chains^{5,20,21} provides a plausible molecular complex which could serve to control p56^{lck} activity and hence to regulate early thymocyte development. Such an association is supported by experiments in which crosslinking of CD3 ϵ on early thymocytes in fetal thymic organ culture results in CD4⁺8⁺ cells which lack V β rearrangements^{22,23}, a phenotype remarkably similar to that seen in thymocytes from mice overexpressing p56^{lck}F505 (ref. 6). It is also possible that a surrogate α -chain polypeptide, analogous to the V-preB and λ 5 molecules that interact with antibody heavy chains in developing B cells^{24–26}, may assist in generating a satisfactory TCR β chain signalling complex. Although further efforts will be required to define the true nature of the receptor structure that links TCR β chain-derived signals to p56^{lck} (and the effector molecules which transmit the p56^{lck}-derived signals to the nucleus) our experiments provide strong support for the view that allelic exclusion at the TCR β locus is mediated by a protein tyrosine kinase, almost certainly p56^{lck}. All evidence suggests that a similar process acts to regulate B-lymphocyte development as well²⁷. □

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An activated Notch receptor blocks cell-fate commitment in the developing *Drosophila* eye

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THE *Notch* locus of *Drosophila melanogaster* encodes a 2,703-amino-acid transmembrane protein required for a variety of developmental processes, including neurogenesis, oogenesis and ommatidial assembly^{1,2}. The Notch protein contains a large extracellular domain of 36 epidermal growth factor-like repeats as well as three Notch/Lin-12 repeats and an intracellular domain with 6 Cdc10/ankyrin repeats, motifs that are highly conserved in several vertebrate Notch homologues³⁻⁹. Truncation of the extracellular domain of the *Drosophila* Notch protein produces an activated receptor, as judged by its ability to cause phenotypes similar to gain-of-function alleles or duplications of the *Notch* locus¹⁰. Equivalent truncations of vertebrate Notch-related proteins have been associated with malignant neoplasms and other developmental abnormalities^{8,11-13}. We present here an analysis of activated Notch function at single-cell resolution in the *Drosophila* compound eye. We find that overexpression of full-length Notch in defined cell types has no apparent effects but that overexpression of activated Notch in the same cells transiently blocks their proper cell-fate commitment, causing them either to adopt incorrect cell fates or to differentiate incompletely. Moreover, an activated Notch protein lacking the transmembrane domain is translocated to the nucleus, raising the possibility that Notch may participate directly in nuclear events.

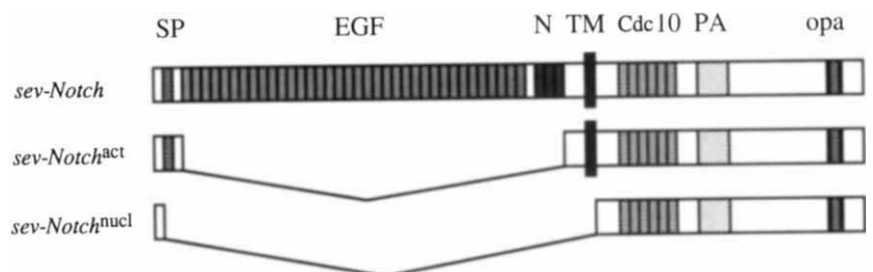
We expressed full-length and truncated Notch proteins under the control of the *Drosophila* *sevenless* gene promoter in transgenic flies (Fig. 1). This promoter is active in the R3, R4 and R7 photoreceptor cell precursors, the four cone cell precursors, and one or two so-called 'mystery cells' of each developing ommatidium^{14,15}. Twenty-seven independent transgenic lines expressing full-length Notch, termed *sev-Notch*, all display wild-type external and internal eye morphology (Fig. 2a-c). Examination of *sev-Notch* flies with anti-Notch antibodies reveals that, in addition to the endogenous Notch staining in all cells of the developing eye imaginal disc, transgenic Notch expression is detected at higher levels in the apical membrane of the disc epithelium¹⁶, in a pattern characteristic of the *sevenless* gene (Fig. 4a). Although overexpressing wild-type Notch in selected cells of the eye disc has no apparent phenotypic consequences, the segregation of bristle and epidermal precursors from equipotent cells of a mosaic notum is strictly correlated to the relative dosages of Notch¹⁷. Such effects in the eye might not be revealed by our analysis, as the *sevenless* promoter is unlikely to be expressed differentially among cells of a single equivalence group.

In contrast to the full-length *sev-Notch* flies, 22 independent transgenic lines bearing the activated *Notch*^{act} construct *sev-Notch*^{act} show moderate roughening of the eye surface, with many ommatidia displaying an unusual 'partial lens' morphology (Fig. 2d, e). As the lens is formed by a secretion from the cone cells, this phenotype suggests that expression of activated Notch in the cone cell precursors delays or otherwise impedes their proper differentiation. Within the retina, 50–80% of the ommatidia in ten *sev-Notch*^{act} transgenic lines examined by sectioning contain only four of the usual six outer photoreceptors R1–6 but have two R7-like cells (Fig. 2f), the remaining ommatidia apparently representing incomplete transformations to the same phenotype. The ectopic R7-like cells express the R7-specific marker *Rh4-lacZ*, confirming their identity as true R7 cells (Fig. 3a). The observed *sev-Notch*^{act} phenotype indicates a block in the normal induction of cells expressing the activated Notch receptor, namely the R3, R4 and R7 precursor cells. Extensive characterization of genetic mutants has shown that when R3 and R4 cannot be determined properly, as in *rough* and *seven-up* mutants, they adopt the R7 cell fate by default^{18,20}. As in *seven-up* retinas, only a subset of the ectopic R7 cells require *sevenless* function for their formation¹⁸ (Fig. 3b). Similarly, when R7 cell commitment is impaired, as in *sevenless*, *bride-of-sevenless*, and *seven-in-absentia* mutants, the R7 cell is transformed into a cone cell^{21,23}. We have no evidence that expression of activated Notch in the 'mystery cells' contributes to the *sev-Notch*^{act} eye phenotype. However, because these cells are normally excluded from the ommatidial precluster early in its formation and are thought to re-enter the population of uncommitted cells¹⁴, they should be unaffected by a transient interference with their ability to undergo cell-fate commitment.

Whereas the Notch^{act} protein has a normal subcellular distribution (Fig. 4b), further deletion of the signal peptide and transmembrane domain produces a protein, Notch^{nuc1}, that is localized to the nucleus (Fig. 4c, d). However, 32 independent transgenic lines bearing this *sev-Notch*^{nuc1} construct have eye phenotypes indistinguishable from *sev-Notch*^{act} (data not shown). The physiological significance of the nuclear Notch localization is unclear, but this observation suggests that the Notch protein may participate directly in nuclear events triggered by Notch receptor activation. Mutant alleles of *Notch* show dosage-sensitive interactions with mutations in two loci that encode nuclear proteins, *mastermind*²⁴ and *Enhancer of split*²⁵, and potential nuclear targeting sequences have been noted in Notch family members⁹.

Because photoreceptor cell precursors are uniquely identifiable by nuclear shape and position as they undergo cell-fate determination, the nuclear localization of the Notch^{nuc1} protein has allowed double-labelling of the *sev-Notch*^{nuc1} eye discs with anti-Notch antibodies and neuron-specific anti-ELAV (*embryonic lethal, abnormal vision* gene product) antibodies^{26,27}. In ommatidial columns 10–20, where R7 cell precursors normally begin neuronal differentiation²⁰, the nuclei of *sev-Notch*^{nuc1} R7 precursors show strong Notch expression but no ELAV expression, consistent with their proposed misspecification as

FIG. 1 Schematic diagram of Notch proteins encoded by constructs present in transgenic *Drosophila*. Polypeptide motifs include the signal peptide (SP), 36 epidermal growth factor-like repeats (EGF), 3 Notch/Lin-12 repeats (N), the transmembrane domain (TM), 6 Cdc10/ankyrin repeats (Cdc10), a putative ATP-binding consensus sequence (PA) and the polyglutamine repeat (opa). The *Notch*-coding fragments present in *sev-Notch* and *sev-Notch*^{act} correspond to those of constructs N and ΔECN, respectively, of ref. 10. *sev-Notch*^{act} and *sev-Notch*^{nuc1} contain deletions of *Notch* gene sequences³ from 948–5,873 bp and 766–6,115 bp, respectively. The *sevenless* gene regula-



tory region contains the promoter and three tandem enhancer elements²⁸.

FIG. 2 Adult eye phenotypes of *sev-Notch* and *sev-Notch^{act}* transgenic animals. Scanning electron micrographs (SEM) of: *a* and *b*, *sev-Notch*, eye; *d* and *e*, *sev-Notch^{act}* eye, anterior at left, dorsal at top. Tangential plastic sections (1 μ m) through the apical retina of: *c*, *sev-Notch* eye with photoreceptor cell identities labelled 1–7; *f*, *sev-Notch^{act}* eye. R7 cells are identifiable by their small-diameter, centrally placed rhabdomeres that extend only through the apical retina. SEM and plastic sectioning were done as described in refs 23 and 21, respectively.

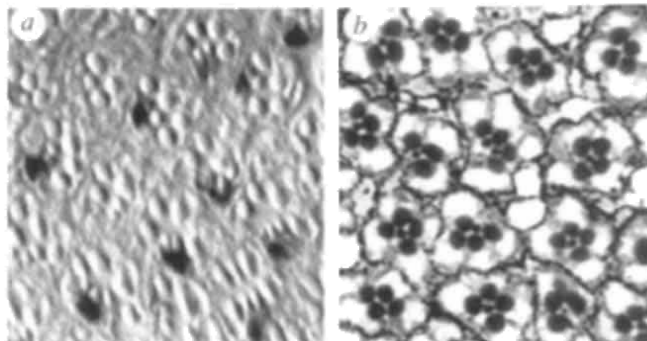
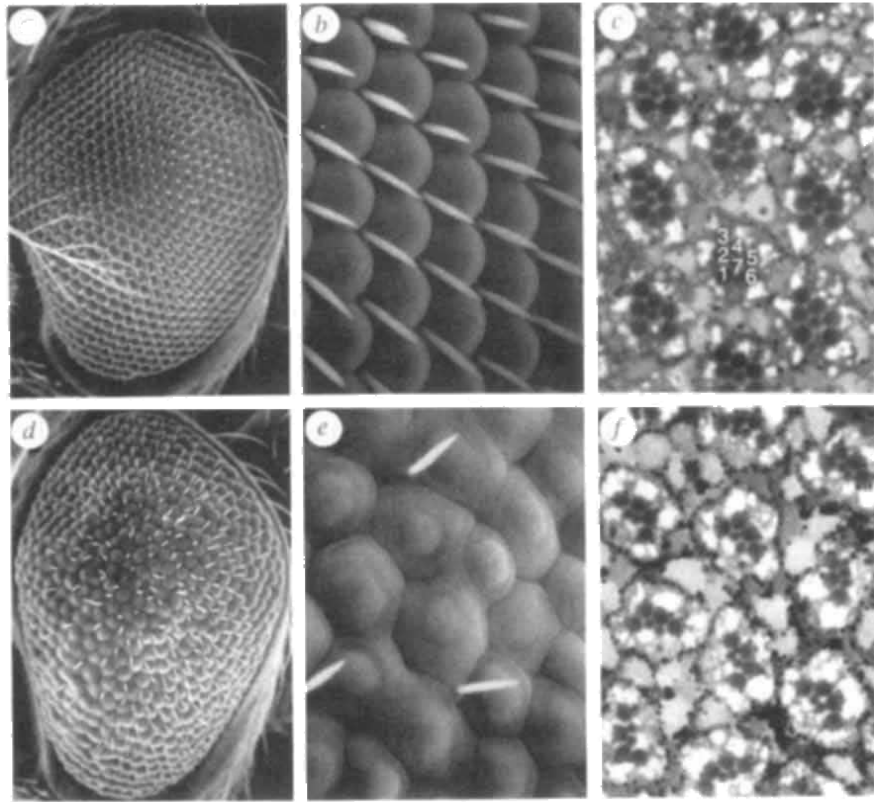


FIG. 3 Properties of ectopic R7 cells produced by *sev-Notch^{act}*. *a*, Nomarski image of a 2- μ m tangential plastic section through an apical retina stained with anti- β -galactosidase antibodies (Promega) from a fly bearing both *sev-Notch^{act}* and the R7-specific rhodopsin promoter construct *Rh4-lacZ²⁹*. Not all R7 cells are stained, because this construct labels ~70% of the R7 cell bodies and a different rhodopsin is expressed in the remaining R7 cells²⁹. *b*, 1- μ m tangential plastic section through the apical retina of a *sevenless^{d2}*; *sev-Notch^{act}* fly, showing that in the absence of *sevenless* gene function, about 50% of the ectopic R7 cells are eliminated. Most ommatidia contain a single R7 cell and ones with two or no R7 cells are seen more rarely. Antibody staining of whole-mount retinæ is described in ref. 30.

non-neuronal cone cells (Fig. 4*e, f*). In contrast, the R3 and R4 precursor cell nuclei show ELAV expression after their early Notch expression has subsided, consistent with their eventual development as R7 neurons. Although the endogenous *sevenless* gene is also expressed weakly in the R1 and R6 precursor cells¹⁴, the *sevenless* gene regulatory sequences used here produced little or no detectable expression in these two cells¹⁵, and we see no nuclear Notch staining in the R1 and R6 precursor cells of *sev-*

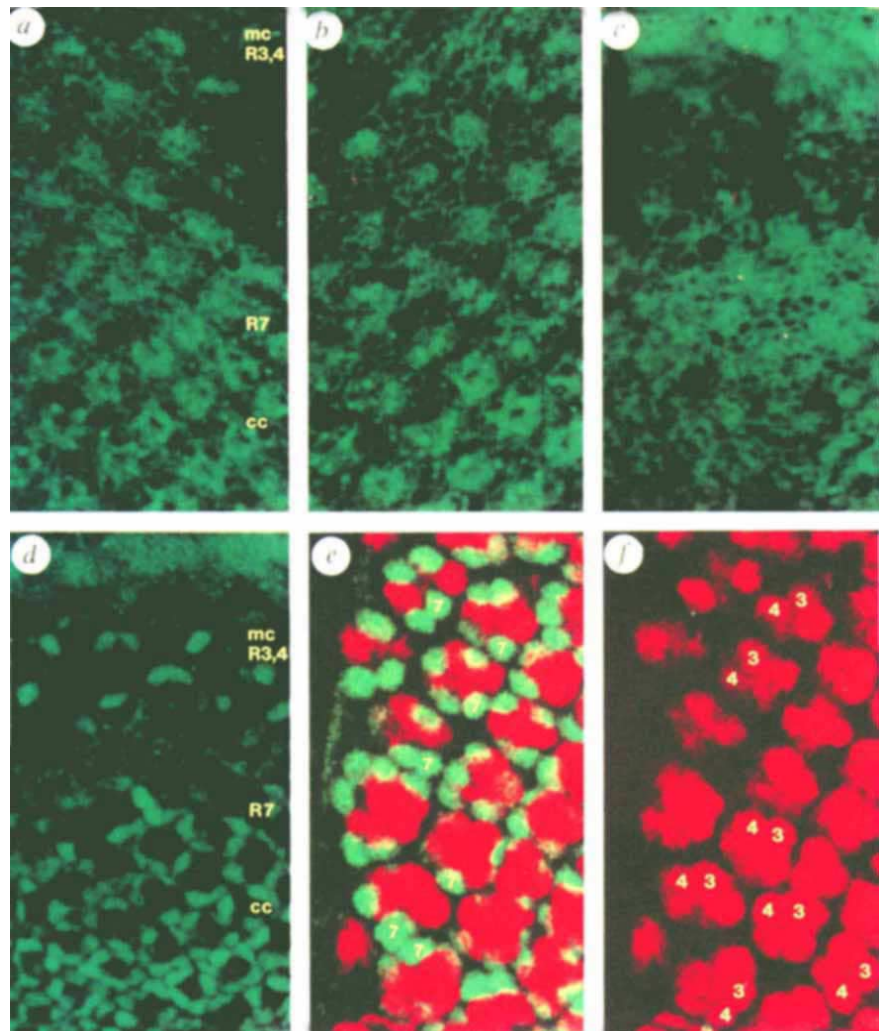
Notch^{nucl} discs. Thus, it is unlikely that these cells contribute to the ectopic R7 cells present in *sev-Notch^{act}* and *sev-Notch^{nucl}* retinæ.

The presence of DNA rearrangements predicted to produce extracellularly truncated Notch proteins in mammalian neoplasms has led to the suggestion that these forms represent activated receptors⁸, as recently demonstrated for *Drosophila* Notch¹⁰. Injection of a similarly truncated *Xenopus* Notch protein into embryos causes the loss of dorsal structures as well as neural and mesodermal hypertrophy¹³. On the basis of this observation, it has been proposed that Notch activation may keep cells in an undetermined state and that activated Notch should cause differentiation delays in *Drosophila*¹³. Our analysis of the function of activated Notch at single-cell resolution in the developing *Drosophila* eye provides direct evidence to support this model. Two classes of cell types were examined: ones that normally commit to neuronal and non-neuronal fates and ones that normally remain uncommitted during the time of the analysis. Transient overexpression of activated Notch receptors in the first class temporarily arrests cell determination, misrouting the R3, R4 and R7 precursor cells into alternative developmental pathways and delaying cone cell differentiation, but it has no effect on the second class of cells. These results, together with those on vertebrate *Notch* homologues, suggest that Notch may act in a general mechanism to maintain the competence of uncommitted cells to respond to later developmental cues. We are testing this idea further by transfecting human *Notch* gene-deletion constructs into cultured cell lines. Moreover, flies with activated Notch receptor constructs may prove useful for genetic screens designed to isolate downstream components of Notch-mediated signalling. □

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FIG. 4 Notch expression and neuronal differentiation in transgenic eye imaginal discs. Mouse monoclonal antibody C17.9C6 directed against the intracellular domain of Notch¹⁶ (green) was used to stain optical tangential sections of third-instar larval eye discs of: a, *sev-Notch*; b, *sev-Notch^{act}*; c, *sev-Notch^{nucl}*; d, a more basal section through the same disc as in c. Sections are oriented with morphogenetic furrow at top and posterior of disc at bottom. *Sevenless* is expressed in mystery cells (mc), R3 and R4 precursor cells, R7 precursor cells, and cone cell precursors (cc). In addition to uniform endogenous Notch staining in apical disc regions of *sev-Notch* and *sev-Notch^{act}*, the apical membranes of *sevenless*-expressing cells are more strongly labelled, producing their characteristic 'butterfly' shapes (R3, R4 cell precursors and mystery cells) in ommatidial columns immediately posterior to the furrow and 'ring' or 'horseshoe' shapes (R7 and cone cell precursors) in more posterior columns¹⁴. The apical region of a *sev-Notch^{nucl}* disc displays only the endogenous Notch distribution pattern (c), but a more basal section through the same disc quadrant reveals strong nuclear staining in expressing *sevenless* cells (d). e, f, Optical tangential section of ommatidial columns 16–20 of a *sev-Notch^{nucl}* eye disc stained with the anti-Notch C17.9C6 antibody (green) and a rat polyclonal antibody against the nuclear antigen ELAV (red). Notch-positive R7 nuclei, labelled '7' in e, do not express ELAV protein. f, The same section labelled with the anti-ELAV antibody alone. ELAV-positive R3 and R4 cells undergoing neuronal differentiation are labelled 3 and 4. Occasional faint Notch staining over these cells corresponds to the basal portions of cone cell nuclei, which have a sausage-like shape and immediately overlie the R1,3,4,6,7 nuclear quintet. Mirror-image symmetry of ommatidia on opposite sides of the dorsal-ventral equator is seen in the lower part of this field; posterior is at left. Antibody staining is described in ref. 30.



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Sphingosine-1-phosphate as second messenger in cell proliferation induced by PDGF and FCS mitogens

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GROWTH signalling networks that use glycerophospholipid metabolites as second messengers have been well characterized^{1,2}, but less is known of the second messengers derived from sphingolipids^{3,4}, another major class of membrane lipids. A tantalizing link between sphingolipids and cellular proliferation has emerged from the discovery that the sphingolipid metabolites sphingosine and sphingosine-1-phosphate stimulate growth of quiescent Swiss 3T3 fibroblasts by a pathway that is independent of protein kinase C^{5,6}. Sphingosine-1-phosphate is rapidly produced from sphingosine and may mediate its biological effects⁶. Furthermore, sphingosine-1-phosphate triggers the dual signal transduction pathways of calcium mobilization^{6,7} and activation of phospholipase D⁸, prominent

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events in the control of cellular proliferation. Here we report that activation of sphingosine kinase and the formation of sphingosine-1-phosphate are important in the signal transduction pathways activated by the potent mitogens platelet-derived growth factor (PDGF) and fetal calf serum (FCS).

To examine the effect of growth factors on sphingolipid metabolites, quiescent Swiss 3T3 fibroblasts were prelabelled with [3 H]serine, a precursor of cellular sphingolipids⁹. Platelet-derived growth factor and fetal calf serum, which are potent mitogens for Swiss 3T3 fibroblasts, induced a rapid and transient increase in [3 H]sphingosine-1-phosphate (SPP), reaching a maximum after 3 min (Fig. 1). Mitogenic concentrations of PDGF induced an identical stimulation of incorporation of both [3 H]serine and 32 P_i into SPP in cells prelabelled to isotopic equilibrium, indicating that the measured changes probably reflect increases in SPP mass. The response was specific for certain mitogens, because epidermal growth factor (EGF), which also stimulates proliferation of these cells^{10,11}, did not induce significant changes. [3 H]sphingosine also increased in the same period in response to FCS and PDGF, but the amount of [3 H]sphingomyelin, which contains a much greater proportion of the total [3 H]serine incorporated into lipids, was not significantly altered (Fig. 1b). Free sphingosine is present at concentrations in the range of 10–100 pmol per 10⁶ cells^{12,13} and may be subject to agonist-mediated regulation¹⁴. However, endogenous SPP has only been detected after treatment of cells with sphingosine^{6,15}. In quiescent Swiss 3T3 fibroblasts, the concentration of SPP is low (16 ± 2 pmol per 10⁶ cells) and increases

in response to PDGF (34 ± 4 pmol per 10⁶ cells) and serum (45 ± 0.3 pmol per 10⁶ cells) to almost the same level formed after addition of mitogenic concentrations (5–10 μM) of sphingosine (40–115 pmol per 10⁶ cells)^{6,8} and is almost the same as the amount taken up when the cells are treated with mitogenic concentrations (1 μM) of SPP (50 pmol per 10⁶ cells)⁶.

The level of SPP in cells is determined by the relative contributions of its formation, mediated by sphingosine kinase¹⁵, and its degradation, catalysed by a pyridoxal phosphate-dependent lyase located in the endoplasmic reticulum^{16,17}. To determine whether a growth-factor-induced increase in intracellular sphingosine is the sole regulatory factor influencing SPP levels, intracellular concentrations of sphingosine were increased about 40-fold by addition of sphingosine. FCS still induced a 1.4-fold increase in SPP within 5 min in sphingosine-loaded cells prelabelled with 32 P_i to isotopic equilibrium. Similarly, when cells were stimulated with FCS and then permeabilized to ATP in a hypotonic buffer containing 50 μM sphingosine and [γ - 32 P]ATP, a significant increase in 32 P-labelled SPP was also observed (Fig. 2a). These results indicate that the increase in SPP may be due not only to the induced increase in sphingosine levels but also to the effects of another possible regulator.

As sphingosine kinase has been proposed to catalyse the rate-limiting step in the metabolism of sphingosine¹⁵, we tested the effects of various growth factors on the activity of this kinase (Fig. 2b, c). FCS and PDGF induce a transient increase in cytosolic sphingosine kinase activity, reaching a maximum within 15 min and returning to basal activity after 1 h (Fig. 2c). In contrast to FCS and PDGF, other mitogens, including bombesin, bradykinin, insulin and EGF, have little or no effect (Table 1). Even a combination of insulin and EGF, which induces marked proliferation of quiescent 3T3 fibroblasts, did not activate sphingosine kinase (Table 1), indicating that the effect is restricted to certain growth-promoting agents.

To investigate the possible role of SPP in cellular proliferation further, we used DL-threo-dihydrosphingosine, a competitive inhibitor of sphingosine kinase^{15,18}. DL-threo-dihydrosphingosine (10 μM) not only inhibited sphingosine kinase activity from 3T3 fibroblasts *in vitro*, it also inhibited the production of SPP induced by a mitogenic concentration of sphingosine (10 μM) by 63 ± 2%, and the initiation of DNA synthesis in response to sphingosine by more than 55%, supporting our previous proposal that SPP mediates the mitogenic effects of sphingosine^{6,8}. This sphingosine kinase inhibitor also reduces

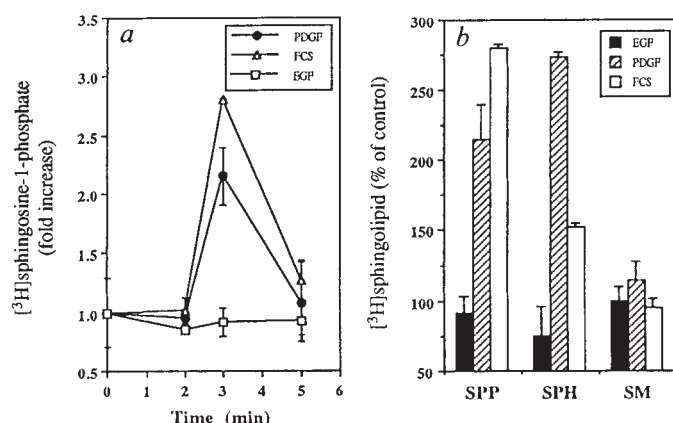


FIG. 1 Effects of growth factors on levels of sphingosine and sphingosine-1-phosphate in Swiss 3T3 fibroblasts. a, Time course of [3 H]sphingosine-1-phosphate formation. b, Effects of FCS, human PDGF or EGF on formation of [3 H]sphingosine, [3 H]sphingosine-1-phosphate and [3 H]sphingomyelin.

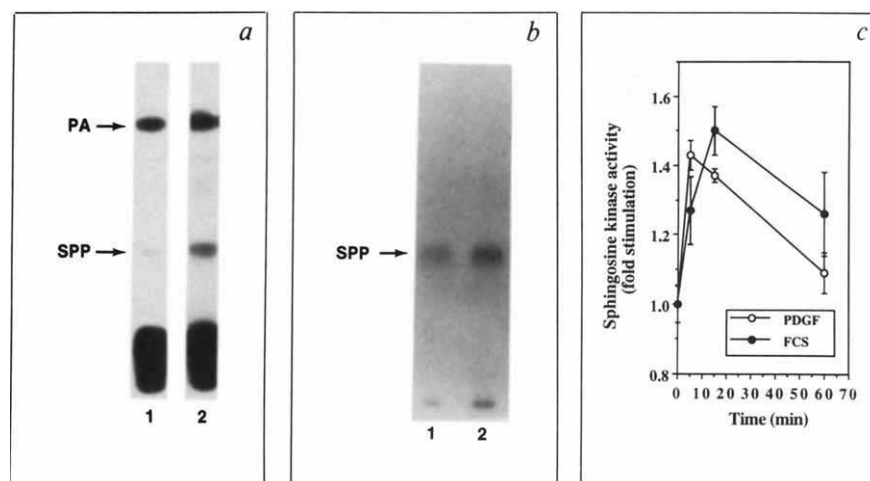
METHODS. Swiss 3T3 fibroblasts from the American Type Culture Collection were subcultured as previously described¹⁰. Confluent and quiescent cells were incubated for 15 h in media containing [3 H]serine (20 μCi ml⁻¹) to label cellular sphingolipids and free sphingosine pools, with 0.5 mM 4-deoxythymidine and 0.1 mM L-canaline to inhibit the pyridoxal-phosphate-dependent SPP aldolase¹⁷. Cells were then stimulated with FCS (10%), human PDGF (10 ng ml⁻¹), EGF (10 ng ml⁻¹) or vehicle for various times, and the lipids were extracted²⁹ and analysed by two-dimensional TLC⁶. Standard SPP and sphingosine were applied with the samples and lipids were visualized using iodine vapours^{6,8}. The principal cellular lipids and sphingolipids were excised from the plate and counted by liquid scintillation spectrometry. The identity of the putative [3 H]SPP was confirmed as described⁶. In a, results were calculated as per cent of the total radioactivity in the lipids and expressed as fold increase of [3 H]SPP formation relative to the untreated cells. Data are the means ± s.d. of three independent experiments. In b, levels of [3 H]sphingosine, [3 H]SPP and [3 H]sphingomyelin were determined after 3 min stimulation and expressed as per cent of the control cells not treated with growth factors. In unstimulated cells, the per cent of [3 H]serine (relative to total lipids) incorporated into sphingomyelin, free sphingoid bases and SPP was 15.8 ± 1.7%, 0.32 ± 0.11% and 0.13 ± 0.019%, respectively.

TABLE 1 Effect of various growth-promoting agents on sphingosine kinase activity

Treatment	Sphingosine kinase activity (fold increase)	[3 H]thymidine incorporation (fold increase)
FCS	1.56 ± 0.06	41.2 ± 1.65
PDGF	1.62 ± 0.01	14.2 ± 0.87
EGF	1.11 ± 0.01	3.7 ± 0.26
EGF + insulin	1.05 ± 0.03	20.5 ± 1.90
Bombesin	1.10 ± 0.01	2.0 ± 0.04
Bradykinin	1.16 ± 0.16	2.7 ± 0.18

Quiescent Swiss 3T3 fibroblasts were incubated in serum-free DMEM and then stimulated with the growth promoting agents for 10 min. Cytosolic fractions were prepared and assayed for sphingosine kinase activity as described in Fig. 2b legend. In unstimulated cells, sphingosine kinase activity was 43.1 ± 4 c.p.m. × 10⁴ mg⁻¹ per 30 min. [3 H]thymidine incorporation was measured in cultures stimulated with the indicated mitogens for 24 h as described^{5,6}. The amount of [3 H]thymidine incorporated into untreated cells was 2 ± 0.3 c.p.m. × 10³ per well. Data are expressed as fold stimulation relative to untreated controls; each value is the mean ± s.d. of triplicate determinations. Concentrations of the mitogenic agents were as follows: FCS (10%), insulin (2 μg ml⁻¹), EGF (20 ng ml⁻¹), PDGF (10 ng ml⁻¹), bombesin (0.6 μM) and bradykinin (1 μM).

FIG. 2 FCS and PDGF enhance the formation of sphingosine-1-phosphate in permeabilized Swiss 3T3 cells (a) and stimulate sphingosine kinase activity (b, c). a, Quiescent cells were treated without (lane 1) or with 10% FCS (lane 2) for 15 min, permeabilized for 20 min at 37 °C in a hypotonic buffer (25 mM HEPES, 5 mM MgCl₂, 5 mM MnCl₂, 20 µM ZnCl₂, 20 µM Na₃VO₄, 10 µg ml⁻¹ leupeptin and aprotinin, and 1 mM PMSF, pH 7.2) with sphingosine (50 µM) and [γ -³²P]ATP (0.5 µM, 20 µCi ml⁻¹). Lipids were extracted, resolved by TLC and visualized using autoradiography⁶. Arrows indicate locations of phosphatidic acid (PA) and SPP. b, After stimulation as for a, cells were washed and lysed by freeze-thawing in 0.1 M phosphate buffer (pH 7.2) containing 10 mM MgCl₂, 20% glycerol, 1 mM mercaptoethanol, 1 mM EDTA, 20 µM ZnCl₂, 1 mM Na₃VO₄, 15 mM NaF, 10 µg ml⁻¹ leupeptin and aprotinin, 1 mM PMSF and 0.5 mM 4-deoxyypyridoxine. Cytosolic fractions were prepared by ultracentrifugation at 105,000g for 90 min. Sphingosine kinase activity was measured in supernatants by incubating with 50 µM sphingosine-BSA complex⁵ and [γ -³²P]ATP (1 mM, 0.2 Ci mmol⁻¹) for 30 min at 37 °C. Labelled lipids were extracted and analysed as described in a. The supernatants contained a specific sphingosine kinase activity because other related lipids, such as galactosylsphingosine, DL-threo-dihydrosphingosine, ceramide, N-hexanoylsphingosine, N-acetylsphingosine, phosphatidyl inositol, and mono-, di- or triacylglycerols are not signifi-



cantly phosphorylated. The formation of the phosphorylated product was dependent on the concentration of protein and sphingosine, showing typical Michaelis-Menten kinetics. The increased activity was attributable to an increase in the V_{max} of the kinase with no apparent alterations in the K_m . c, After stimulation with FCS (10%) or human PDGF (10 ng ml⁻¹) for the times indicated, cytosolic fractions were prepared and sphingosine kinase activity determined. Data are expressed as fold increase over untreated controls.

DNA synthesis induced by PDGF and serum by 40 and 44%, respectively (Table 2), a result that is consistent with the multiplicity of signal transduction systems known to be stimulated by these growth factors². The effects of this inhibitor on DNA synthesis are specific because it did not abrogate cellular proliferation of quiescent Swiss 3T3 fibroblasts induced by EGF, which seems to act independently of SPP formation. Furthermore, under conditions where it markedly inhibited DNA synthesis, DL-threo-dihydrosphingosine completely eliminates the production of SPP elicited by PDGF.

Protein kinase C is a pivotal regulatory enzyme in PDGF- and serum-induced cellular proliferation^{2,19}, but the mitogenic effects of sphingosine and SPP are clearly independent of protein kinase C^{5,6}. DL-threo-dihydrosphingosine inhibits DNA synthesis induced by PDGF and FCS to the same extent in cells rendered deficient in protein kinase C by prolonged exposure to 12-O-tetradecanoylphorbol-13-acetate (TPA)^{5,6}. Furthermore,

the inhibition of DNA synthesis by the combination of H-7, an inhibitor of protein kinase C²⁰, and DL-threo-dihydrosphingosine was almost additive (Table 2), as might be expected for inhibitors that block different signal transduction pathways.

As our knowledge of the mechanisms of cell growth regulation has increased, it has become apparent that there exist intracellular second messengers that have not been identified. Polyphosphoinositide hydrolysis is not required for growth factor-induced mitogenesis^{21,24}, and it is the activation of phospholipase D rather than phospholipase C that correlates with mitogenesis induced by PDGF²⁵. Furthermore, PDGF may stimulate the formation of another intracellular second messenger capable of releasing calcium from intracellular sources, because PDGF has virtually no effect on inositol 1,4,5-trisphosphate levels in Swiss 3T3 fibroblasts²⁶. Our data provide the first clues to the identity of a possible missing link between the plasma membrane (where the growth factor receptors are found), intracellular calcium stores, and cellular proliferation. SPP has properties that make it a suitable candidate to function as this link: it elicits diverse cellular responses^{6,8,27}; its turnover is extremely rapid^{15,16}; its level in the cell is low and increases rapidly and transiently in response to FCS and PDGF; and it releases calcium from internal stores but independently of inositol trisphosphate^{6,7}. Finally, SPP might act in a positive feedback loop to amplify the cascade of events following receptor stimulation as a result of its effect on phosphatidic acid levels⁸, which links growth factor signalling to cellular *ras* activity²⁸. □

TABLE 2 Effects of DL-threo-dihydrosphingosine and H-7 on DNA synthesis induced by various growth factors

Inhibitors	[³ H]thymidine incorporation (c.p.m. per well)		% Inhibition		
	None	DHS	DHS	H-7	DHS + H-7
Stimulants					
None	2,430 ± 180	2,300 ± 210*	NS	NS	NS
FCS	85,070 ± 5,390	47,680 ± 3,000†	44	40	68
PDGF	68,960 ± 3,970	41,730 ± 290†	40	19	58
EGF	22,350 ± 390	21,720 ± 820*	NS	ND	ND

[³H]thymidine incorporation was measured^{5,6} in quiescent cultures of Swiss 3T3 cells exposed to the indicated growth factors in the absence or presence of 10 µM DL-threo-dihydrosphingosine (DHS) and/or H-7 (1-(5-isoquinolylsulphonyl)-2-methylpiperazine). Each value is the mean ± s.d. of triplicate determinations from a representative experiment. Similar results were obtained in at least five other experiments. Data are expressed as per cent inhibition relative to cells cultured in the absence of inhibitors. Concentrations of mitogenic agents were as follows: FCS (10%); PDGF (10 ng ml⁻¹); EGF (20 ng ml⁻¹) in the presence of insulin (2 µg ml⁻¹). NS, no significant inhibition; ND, not determined. * $P \geq 0.5$; † $P < 0.002$.

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Cell-cycle control of a large-conductance K^+ channel in mouse early embryos

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THERE have been few investigations into the role of ion channels in mammalian early embryonic development^{1–4}, despite studies showing that changes in ion channel activity accompany the early embryonic development of non-mammalian species^{5–7} and the proliferation of mammalian cells^{8–12}. Here we report that a large-conductance, voltage-activated K^+ channel is active in unfertilized mouse oocytes but is rarely observed in later embryos. The channel activity is linked to the cell cycle, being active throughout M and G1 phases, and switching off during the G1-to-S transition. These changes in channel activity are accompanied by corresponding shifts in membrane potential. Inactivation of the channel during S/G2 can be prevented by exposing the oocytes to dibutyryl cyclic AMP or forskolin, an activator of adenylyl cyclase. Inhibition of protein synthesis with puromycin did not prevent inactivation of the channel at the end of G1 or its subsequent reactivation at the end of G2, indicating that the channel activity is not regulated by mitosis-promoting factor or cyclins.

The predominant ion channel type in cell-attached patches on unfertilized mouse oocytes has an inwardly rectifying current-voltage relation (Fig. 1b), with a limiting single-channel conductance of 241 ± 8 pS (mean $\pm$ s.e.m., $n = 23$) and a reversal potential of ~ 60 mV (corresponding to a transmembrane potential of $+16$ mV), and is activated by depolarization (Fig. 1a, c). The channel is not active in excised patches, so its ion selectivity was determined in cell-attached patches. The addition of 150 mmol l^{-1} KCl (but not NaCl) to the pipette solution depolarized the channel's reversal potential by 17 mV, as expected for a K^+ -selective channel (Fig. 1d).

In the unfertilized oocyte, the channel was observed in 83% of cell-attached patches (Table 1). After fertilization, its incidence was reduced to less than 10% (Table 1) and the plasma membrane potential depolarized (Table 1; ref. 2). Because the unfertilized oocyte is arrested at metaphase of the second meiotic division, these changes could be attributable either to the resumption of the cell cycle or to the activation of development. The use of nocodazole to destabilize microtubules and arrest fertilized embryonic cells in mitosis caused the 240 pS K^+ channel to reappear (Table 2). This reappearance was attributable to arrest in metaphase rather than to the presence of nocodazole because (1) exposure of interphase cells to nocodazole did not increase channel incidence, and (2) the channel was observed in 8 of 12 cell-attached patches on fertilized zygotes that had entered mitosis in the absence of nocodazole.

The relationship of 240 pS channel activity to the cell-cycle phases was investigated more carefully in the first cell cycle in which the appearance of pronuclei can be used as a reference point¹³. The channel incidence was high (86%) 1.5 hours after the formation of pronuclei (7.5 h post-fertilization), but declined to a minimum of 7% 11.5 h after the formation of pronuclei (G1 to S transition), rising again to 79% 15.5 h after the formation

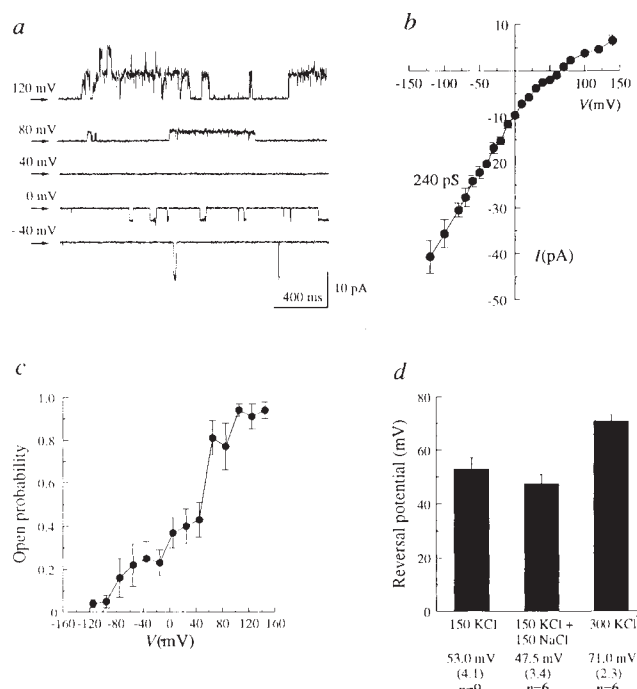


FIG. 1 Properties of the 240 pS K^+ channel in cell-attached patches on unfertilized oocytes. **a**, Representative recordings. **b**, Single-channel current-voltage relation. Points are means $\pm$ s.e.m. of 2 to 27 separate experiments. **c**, Effect of transmembrane potential on the open probability. Points are means $\pm$ s.e.m. of 3 to 23 values grouped in bins of 20 mV. **d**, Ion selectivity of the 240 pS channel in cell-attached patches studied using pipette solutions that altered the Nernst equilibrium potential for K^+ and Na^+ independently. The reversal potential for the current-voltage relation of the 240 pS channel is shown for separate experiments when the pipette solution contained 150 mM KCl, 150 mM KCl plus 150 mM NaCl, or 300 mM KCl. The mean reversal potentials are given below with the s.e.m. in brackets.

METHODS. Superovulated oocytes and embryos were obtained from 3–4-week-old QS mice as described in ref. 28. H6 medium²⁸ was used for handling oocytes/embryos and either T6 medium²⁸ or CZB medium²⁹ was used for culture in 5% CO_2 . Cumulus cells were removed from oocytes by brief treatment with hyaluronidase (0.01%) and the zona pellucida removed from oocytes by minimal exposure to chymotrypsin (0.001%) in H6 and from embryos by pronase²⁸. In patch-clamp experiments, the oocytes were bathed in H6 medium (no BSA) and the pipette solution contained (in mM) KCl (140), HEPES (10), EGTA (0.5), glucose (10) and EDTA (0.01), pH 7.2. The applied voltage is indicated to the left of each record and arrows indicate zero-current levels. All potentials are expressed as bath relative to the pipette and are the clamped patch transmembrane potentials relative to the resting cell membrane potentials. Currents are defined as positive when the direction of flow of positive charge is from the cell into the pipette.

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TABLE 1 Incidence of the 240 pS channel in cell-attached patches and mean whole-cell potentials of oocytes and embryos at various stages

Channel type	Unfertilized oocyte (%) (n = 122)	Fertilized zygote (%) (n = 61)	Two-cell embryo (%) (n = 29)	Four-cell embryo (%) (n = 16)	Eight-cell embryo (%) (n = 16)	Blastocyst (%) (n = 13)
240pS	83	10	3	6	0	0
Mean cell potential (mV $\pm$ s.e.m.)	-44.1 $\pm$ 1.2 (n = 56)	-31.8 $\pm$ 1.0 (n = 41)	-28.0 $\pm$ 2.2 (n = 13)	-24.1 $\pm$ 3.7 (n = 13)	-22.6 $\pm$ 2.2 (n = 20)	-31.0 $\pm$ 7.0 (n = 2)

Channel incidence is expressed as the percentage of patches in which activity of the 240 pS was observed, seal resistance was greater than 10 G Ω and the patch lasted for at least 5 min. The patch was clamped at transmembrane potentials from 200 to 80 mV for ~30-s periods to promote channel opening (Fig. 1c). Pipettes with tip resistances between 2 and 5 M Ω were used. Values are given as means $\pm$ s.e.m. for *n* separate experiments.

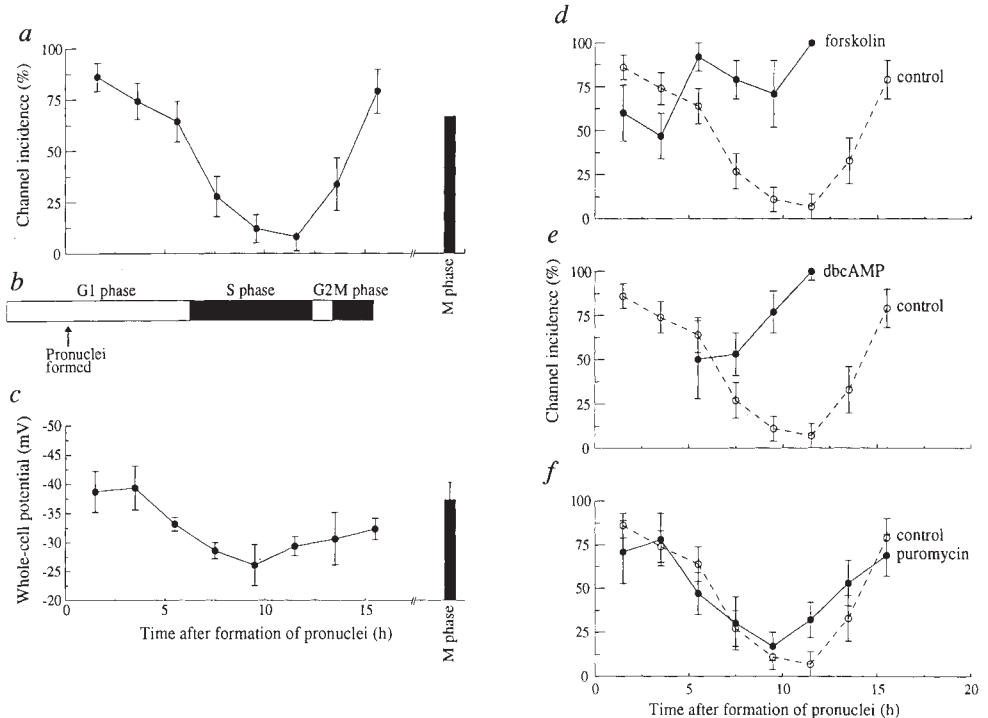
of pronuclei (G2 to M transition) (Fig. 2a). These changes in channel incidence were accompanied by the expected changes in whole-cell potential (Fig. 2c). The gradual transitions in channel incidence probably reflect the variability of up to 2–3 h in progression through the cell cycle observed among fertilized mouse oocytes¹³. This is confirmed by studies on cell-attached patches from two-cell embryos, which have a very short G1 and a long G2¹⁴, and in which no channel activity was detected in S (*n* = 7) or in G2 (*n* = 9). That the channel is inactive during S phase, but active during G1 and M phases, seems to be in conflict with the absence of the channel from cell-attached patches from untimed cleaving embryos (Table 1), but is in fact consistent with the predominance of the S and G2 phases in the cell cycles of the cleaving mouse embryo^{14,15}.

The channel is not controlled by cytosolic Ca²⁺ (refs 16–18), because use of a Ca²⁺ ionophore (ionomycin) to increase the free Ca²⁺ inside the zygotes (as demonstrated with Fura-2; M.L.D., unpublished experiments) failed to activate it. The rapid inactivation of the channel on patch excision suggested that a cytosolic process, such as the maintenance of a short-lived phosphorylated state, might be essential for its activity. Both forsko-

lin, which activates adenylyl cyclase, and dibutyryl cAMP, in concentrations that did not block progression through the cell cycle, prevented the disappearance of the K⁺ channel during S/G2 of the first cell cycle (Fig. 2d, e). Use of the protein synthesis inhibitor puromycin, which prevents entry of zygotes to M phase¹³, failed to prevent the inactivation of the K⁺ channels, neither did it prevent their subsequent reactivation at the time they would have been expected to enter M phase (Fig. 2f). Thus reactivation of the channel is not dependent on entry of nuclei into M phase.

Although the K⁺ channel described here is similar in conductance and gating properties to the 'maxi-K' channel observed in many other tissues¹⁹, it is not blocked by charybdotoxin, nor is it sensitive to cytosolic Ca²⁺. The channel's conductance, voltage-sensitivity, insensitivity to Ca²⁺ and inactivation on patch excision distinguish it from the K⁺ channels reported in hamster²⁰ and mouse oocytes²¹, neither of which appear to change with the cell cycle. The role of the 240 pS K⁺ channel in the cell cycle of the mouse early embryo is unknown. K⁺ channel blockers inhibit proliferation of lymphocytes^{10,22,23} and of Schwann cells⁹, perhaps by disrupting volume control²⁴. Our observation that

FIG. 2 a–c, The incidence of the 240 pS channel at different times during the first cell cycle of the fertilized oocyte. Zona-free fertilized oocytes were patch-clamped at known times after pronuclei formation. In a, each point represents a bin width of 2 h, starting from 0.5 h after formation of the pronuclei, and the channel incidence is shown as a percentage of the total number of patches. Each bin contained between 14 and 28 patches. b, Roughly corresponding stages of the cell cycle¹³. c, Cell potential measured immediately after achieving the whole-cell configuration. The bars in a and c represent the channel incidence and mean cell potential, respectively, for zygotes in M phase (as shown by the absence of pronuclei). The cell potential during M phase was for nocodazole-arrested oocytes -35.9 ± 1.6 mV (*n* = 15) compared with -37.3 ± 2.9 mV (*n* = 6) for non-arrested oocytes. These potentials are higher than those reported by Peres³⁰. This is possibly due to the use by Peres of solutions containing 20 mM Ca²⁺ to enhance seal formation (M.L.D., unpublished results). d–f, The effects of cAMP and protein synthesis inhibition on incidence of the 240 pS channel. Zygotes were cultured in medium containing either 10 or 100 μ M forskolin (d; *n* = 2 to 15 patches per bin), 1 mM dibutyryl cAMP (e; *n* = 5 to 15 patches per bin) or 10 μ M puromycin (f; *n* = 7 to 23 patches per bin) and patch-clamped at known times after formation



of the pronuclei (filled circles). Open circles represent control channel incidences. Each point represents a bin width of 2 h, starting from 0.5 h after formation of the pronuclei, and the channel incidence is shown as a percentage of the total number of patches, with standard error bars calculated assuming a binomial distribution.

TABLE 2 Effect of nocodazole on the incidence of the 240 pS channel in cell-attached patches on unfertilized oocytes and mouse early embryos

Embryo stage	M-phase	Nocodazole present	Channel incidence		
			Channel present	Total patches	%
Unfertilized oocyte	yes	—	22	26	85
	yes	+	18	20	90
Fertilized zygote	no	—	1	23	4
	no	+	1	11	9
	yes	—	8	12	67
	yes	+	16	26	62
Two-cell embryo	no	+	0	9	0
	yes	+	4	6	67
Four-cell embryo	no	+	0	10	0
	yes	+	7	10	70
Eight-cell embryo	no	+	0	4	0
	yes	+	5	6	83

Channel incidence was determined as described in the legend to Table 1. Zygotes were cultured in medium T6 (ref. 28) + BSA (4 mg ml⁻¹) in the presence or absence of 10 µM nocodazole (Aldrich, Milwaukee, USA) at 37 °C in 5% CO₂ until the cell entered mitosis, as indicated by the disappearance of nuclear membranes. Values are given as means ± s.e.m. for *n* separate experiments.

dibutyl cAMP and forskolin maintain activity of the 240 pS channel but do not interfere with division indicates that channel inactivation during S/G2 is not essential for progress of embryos through the cell cycle.

The nature of the link between the cell cycle and the activity of the 240 pS K⁺ channel is not clear. In loach eggs, there is a stretch-activated K⁺ channel that is activated during cell cleavage⁵, but stretch activation of the mouse channel seems unlikely (M.L.D., unpublished results, and ref. 25). cAMP activates the stretch-activated K⁺ channel in loach embryos⁶ and the Cl⁻ conductance in B lymphocytes⁸, both of which vary during the cell cycle. Our findings also suggest that cAMP may form part of the link between the cell cycle and the 240 pS K⁺ channel, but the slow onset and sustained action of cAMP differ from the rapid but transient action observed in these other systems, suggesting that cAMP may be acting by different mechanisms. By analogy with the cyclical reactivation of a Cl⁻ conductance in *Botlenia* embryos during mitosis²⁶, the 240 pS K⁺ channel may be regulated by insertion of the channel protein into and removal from the plasma membrane. Our data do not distinguish between changes in ion channel numbers and in channel open probability, but the delayed effect of dibutyl cAMP on activation of the K⁺ channel is consistent with a process, such as vesicle insertion into the egg membrane, being involved.

We can also conclude that the reactivation of the K⁺ channel during M and G1 phases is not dependent on the activation of mitosis-promoting factor (MPF). Puromycin inhibits protein synthesis and hence the synthesis of cyclin B, a component of MPF. It also prevents two zygotic markers of MPF activity: pronuclear membrane breakdown and the rephosphorylation of a 35K protein in murine eggs²⁷. Presumably the reactivation of the K⁺ channel in the presence of puromycin is either an earlier event in the pathway of cyclin synthesis or is on a parallel, independent cell-cycle pathway, perhaps similar to the cytoplasmic clock observed in mouse eggs²⁵. □

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Residues in the TATA-binding protein required to mediate a transcriptional response to retinoic acid in EC cells

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THE eukaryotic TATA-binding protein TBP, which is required for transcription by RNA polymerase II, is tightly associated with a particular set of factors in the TFIID complex^{1,5}, and as such provides a target for transcriptional regulation exerted by upstream factors. An embryonic carcinoma (EC) cell-specific activity like that of the viral factor E1A has been implicated in the mediation of transactivation from the retinoic acid receptor to human TBP, but yeast TBP cannot perform this function⁶. Using TBP mutants with an altered TATA-box-binding specificity⁷, we show here that yeast TBP can mediate transcriptional activation in mammalian cells and that its inability to convey retinoic acid-dependent transactivation in EC cells is due to specific residues in its core region. These residues preclude a functional association with the cellular E1A-like activity. TBP is thus a target for retinoic acid-dependent transactivation in EC cells by providing a surface for interaction with the EC cell-specific E1A-like activity.

To test whether the inability of yeast TBP to mediate retinoic acid-dependent transactivation in EC cells could be due to an inability to interact with either mammalian TBP-associated factors (TAFs) or with the E1A-like activity (E1A-LA) that is specific to EC cells, we cotransfected yeast TBP and the retinoic acid receptor (RAR) together with an RAR-β2 promoter reporter construct in the E1A-transformed cell line 293 (Fig. 1a). We found that yeast TBP can support retinoic acid-dependent transactivation as efficiently as its human counterpart, indicating

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that yeast TBP is able to interact efficiently with mammalian factors. This finding is in line with the observation that yeast TBP can specifically bind E1A *in vitro*, although with a lower efficiency than human TBP⁸. Similar results were obtained in COS and EC cells upon co-expression of the 13S but not 12S form of E1A (data not shown).

The finding that expression of yeast TBP boosts the level of RAR- β 2 transcription when coexpressed with E1A could be due to titration of inhibitory factors such as NC1, NC2 and Dr1 (refs 9,10), or it could indicate that yeast TBP interacts with E1A. To clarify this point, we used yeast and human TBPs with altered DNA-binding specificities (TBP-spm3). These TBP-spm3 proteins permit transcription from promoters containing the mutated TATA box, TGTA AAA, in yeast⁷. Wild-type and mutant human or yeast TBPs were analysed for their ability to cooperate with RAR in transactivation of reporter genes that contain either TATA AAA (reporter M2)⁶ or TGTA AAA (reporter M1). Cotransfection of wild-type TBPs and E1A enhanced transcription only from M2 and not from the TGTA AAA-containing reporter. By contrast, coexpression of human or yeast TBP-spm3 results in transactivation on both promoter templates (Fig. 1b, c). Thus, the transfected human as well as yeast TBP-spm3 derivatives mediate transactivation of the promoter on binding to the mutated TATA-box element. Yeast TBP can also function in conjunction with other transactivators such as GAL-VP16 (ref. 11) even in the absence of co-transfected E1A (Fig. 1d). GAL-VP16 does not function with recombinant TBP *in vitro* but requires

the TFIID complex of TBP and TAFs¹². Taken together, these results indicate that yeast TBP can interact with mammalian TAFs *in vivo*. The high degree of conservation in the core domain of TBP among different organisms can be seen as an evolutionary constraint due to the importance of the TBP/TAF interaction. But, unlike the TFIID activity from higher organisms, the yeast TFIID activity fractionates as a single protein on phosphocellulose column chromatography^{13,14}. Yeast TBP may also have several TAFs that are more loosely associated and thus do not survive biochemical fractionation¹⁵.

It has been reported that the basic domain of TBP is essential and sufficient to interact with E1A *in vitro*¹⁶. To establish the region of the human TBP core that is necessary for interaction with E1A-LA, we generated human/yeast TBP-spm3 chimaeras. We discovered that the N-terminal region of the core (section C1) of human TBP was required to mediate transactivation by the EC cell-specific E1A-LA (Fig. 2, and data not shown). Next, the C1 sequence was subdivided into three regions, a, b and c, of which b and c encompass the basic domain that has been reported to bind E1A *in vitro*¹⁶. Cotransfection of these TBP chimaeras with M1 demonstrated that the human C1a region contains the sequence critical for interaction with the E1A-LA (Fig. 2). All TBP chimaeras were able to mediate retinoic acid-dependent transactivation in conjunction with co-expressed viral E1A (13S) (data not shown). Western blot analysis confirmed that the expression of all TBP chimaeras was comparable (data not shown).

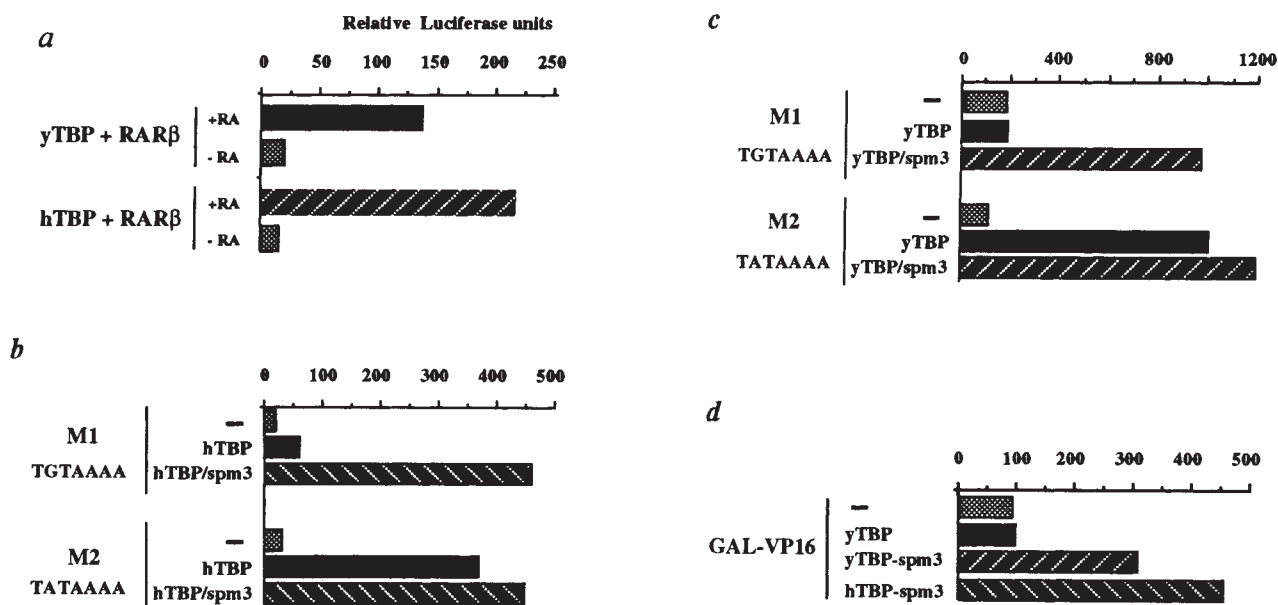
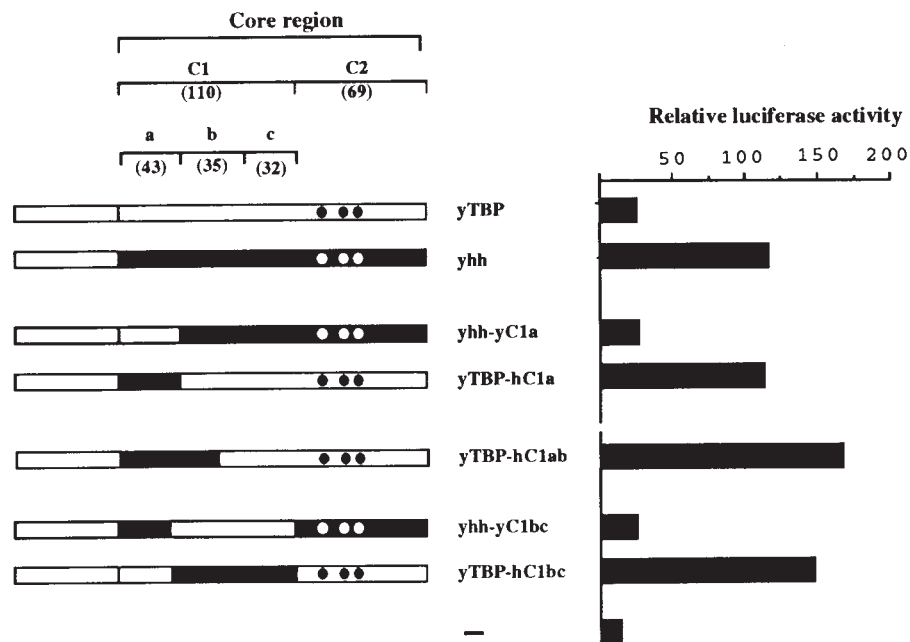


FIG. 1 Yeast TBP (yTBP) supports transactivation in mammalian cells. **a**, E1A-dependent transactivation of the RAR- β 2 promoter with human or yTBP in 293 cells. RA, retinoic acid. **b**, Transfections of wild type and TBP-spm3 from either human or c, yeast with reporter constructs M1 (TGTA AAA) and M2 (TATA AAA) in COS cells. **d**, Transfection of GAL-VP16 expression vector and wild-type yTBP and mutant yeast and human TBP-spm3 with 5 $\times$ (GAL4)-M1-luc in COS cells.

METHODS. 293 and COS cells were grown in DMEM medium containing 10% fetal calf serum, non-essential amino acids and penicillin-streptomycin. 24 Hours before transfection, cells were seeded at a density of 1×10^6 per 6-cm dish. Cells were transfected by calcium phosphate coprecipitation with a total of 9 μ g DNA per dish (293 cells), which included 3 μ g of the indicated TBP expression plasmid, 5 μ g luciferase reporter plasmid containing the RAR- β 2 promoter, 0.5 μ g of the RSV-CAT internal control and 0.5 μ g hRAR- β . In addition, COS cells received 1 μ g of indicated E1A construct per dish (**b** and **c**). The total quantity of DNA in all control transfections was kept constant by adding the appropriate amount of the empty expression vector pSG5 (ref. 27). After overnight exposure of the cells to the DNA-calcium phosphate coprecipitate, they were fed with fresh medium containing 1×10^{-6} M

retinoic acid. After 24 h, cells were collected and whole-cell extracts prepared for determination of luciferase and chloramphenicol acetyltransferase activities and for western blotting⁶. Luciferase values are normalized for RSV-CAT expression and represent the average of at least two independent experiments, in which each transfection was done in duplicate. M4 contains the RAR- β 2 promoter sequence from -124 to +14 in front of the luciferase gene and an additional XhoI site inserted at position -31 (ref. 6). M1 and M2 receptor constructs were created by replacing the XhoI/XbaI fragment in M4 containing the wild-type TATA-box sequence with the oligonucleotides containing either the adenovirus major late TATA box (5'-TCGATATAAGGCT-3'; M2) or the mutated TATA box (5'-TCGATGTAAAGGCT-3'; M1). The human and yTBP specificity mutants⁷ were subcloned as EcoRI/BamHI fragments into the expression vector pSG5. The 5 $\times$ GAL-M1-luc reporter was constructed by replacing the RAR- β 2 promoter (nucleotides -31 to -124) from the M1 reporter by a 5 $\times$ repeated GAL4 binding site. GAL-VP16 consists of the GAL DNA-binding domain (amino acids 1-74) fused to the transactivation domain of VP16 (amino acids 410-489; kindly provided by F. Stewart).

FIG. 2 The TBP core region necessary for mediation of transactivation by E1A-LA. Cotransfections of the TBP-spm3 chimaeras and M1 reporter plasmid in EC cells; similar results were obtained for M2 and M4 reporter constructs (data not shown). A schematic diagram of TBP core chimaeras is indicated. All TBP constructs contain the spm3 mutation in the C2 region⁷ (indicated by dots). The number of amino acids within the core subregions are given in brackets. **METHODS.** RAC65 cells were maintained in DMEM:EMEM medium (1:1) containing 7.5 % ox serum, non-essential amino acids and penicillin streptomycin. Cells were seeded at a density of 5×10^5 per 6-cm dish and transfected as described for Fig. 1. Proteins from whole-cell extracts were separated by 12 % SDS PAGE, transferred to a PVDF membrane, blocked and washed in PBS buffer containing 1 % Triton X-100 and 1 M NaCl. Incubation with antibody was in the same buffer containing 5 % skimmed milk powder. A rabbit polyclonal antibody raised against the N terminus of yTBP (kindly provided by S. Buratowski) and an alkaline phosphatase-linked anti-rabbit immunoglobulin were used as primary and secondary antibodies, respectively. Protein-antibody complexes were visualized using alkaline phosphatase detection reagents as recommended by the supplier (Promega). All TBP-spm3 chimaeras were fused to the yeast N-terminal sequence for identification by an antibody against this region on western blots. The TBP constructs yhh, yhh and yhy²⁸ were subcloned into the pSG5 expression vector as *EcoRI*/*Bam*HI fragments. The C1 sequence of the TBP core domains was subdivided by introducing *Fsp*I and *Taq*I sites at amino-acid positions 203 and 238



in hTBP and at positions 104 and 139 in yTBP. A unique *Fsp*I site was introduced into both human and yTBP with the oligonucleotide 5'-GCTCTCTTATGCGCATGATTACAGCAGCAA-3'. The primers 5'-GTAC-AACTCGAGCATATTT-3' and 5'-GGATAATTCGAGCATATTT-3' were used to introduce *Taq*I sites at analogous positions in human and yTBP, respectively. Chimaeras were constructed using the novel *Taq*I and *Fsp*I sites and the *Xba*I site at the C1/C2 boundary (amino-acid positions 270 and 172 in human and yeast TBPs, respectively)²⁸.

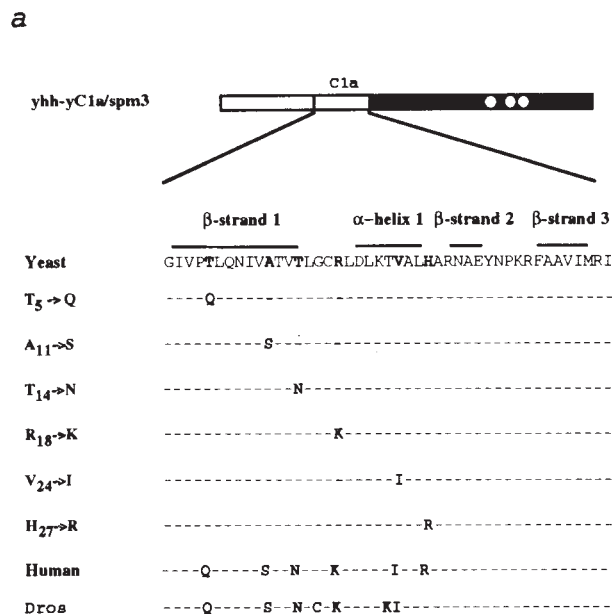
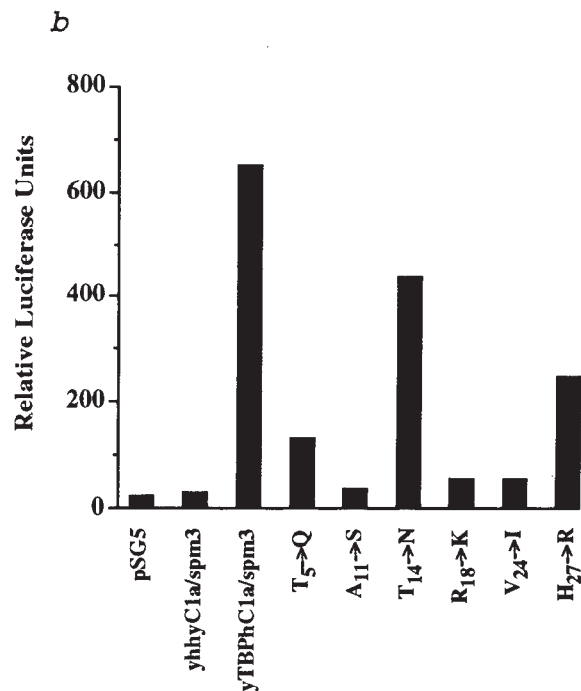
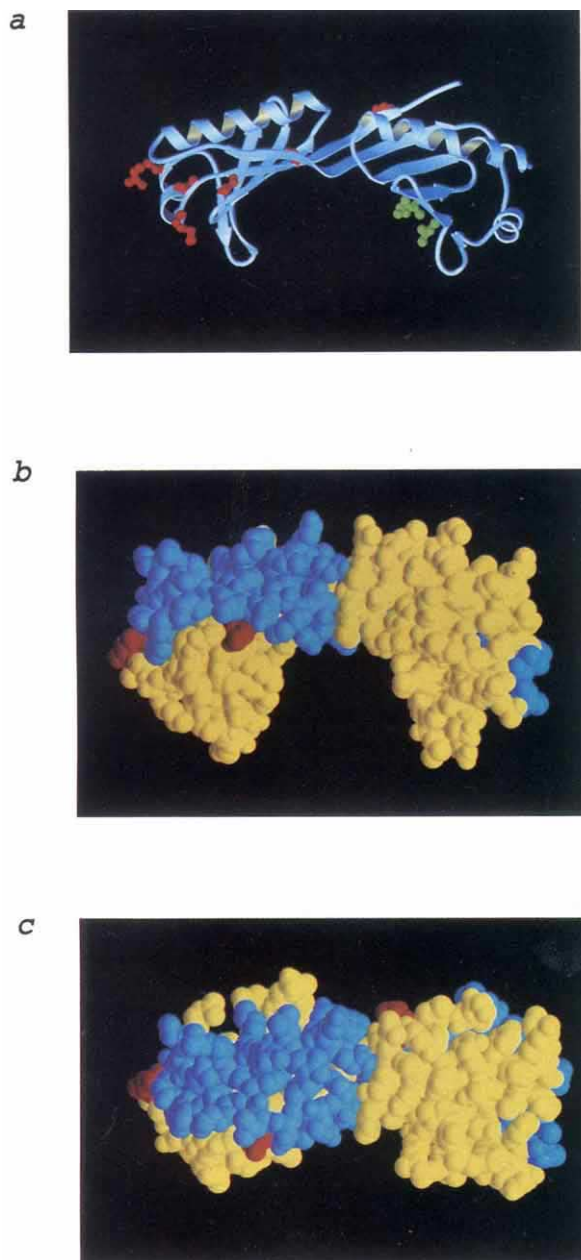


FIG. 3 Amino acids in the C1a region of hTBP required for interaction with the E1A-LA. **a**, Schematic representation of the yhh-yC1a/spm3 TBP chimaera. The positions of the single amino-acid reversions introduced in the yC1a region are depicted. Numbering refers to the amino-acid position in the core region starting from the sequence GIVP which denotes the beginning of the core domain (glycine, amino acid number 1, is located at positions 157, 57 and 174 in human, yeast¹⁴ and *Drosophila*²⁹, respectively). **b**, Cotransfections in RAC65 cells³⁰ of the



TBP mutants depicted in **a** in conjunction with the M1 reporter construct were carried out as described for Fig. 1.

METHODS. Amino-acid substitutions were introduced by PCR-mediated site-directed mutagenesis. The following primers were used; T₅ → Q, 5'-ATTGTTCCACAGCTACAAAAC-3'; A₁₁ → S, 5'-ACATTGTGTCAACTGTGA-3'; T₁₄ → N, 5'-GCAACTGTGAATTTGGGG-3'; R₁₈ → K, 5'-TGGGGTGCAGTT-AGATCT-3'; V₂₄ → I, 5'-TGAAACAATTGCGCTAC-3'; H₂₇ → R, 5'-TTG-CGCTACGTGCCCGTA-3'.



◀ FIG. 4 Computer-generated models of human TBP. Human TBP models were generated by an automated model-building (WHAT IF) program³¹ based on the coordinates of *A. thaliana* TBP¹⁷. a, Ribbon drawing of a representation of the three-dimensional structure of hTBP viewed perpendicular to the 2-fold symmetry axis; α -helices and β -strands are drawn in blue. The amino acids in the C1a region that differ between human and yTBP are shown in red, and the residues that confer altered TATA-box binding specificity are shown in green. Computer-generated atom spherical model of a frontal (b) and top view (c) of hTBP. The atoms are colour-coded as follows: blue, the minimal region of TBP required for interaction with E1A *in vitro*¹⁶; red, the amino acids in the C1a region that are critical for the E1A-LA interaction; the remainder of the molecule is shaded in yellow.

α -helix 1 and T5→Q swaps in β -strand 1 partially restored this function (Fig. 3b). We conclude that although these residues each contribute to the transactivation effect, their concerted action is required to potentiate full transactivation activity. This is supported by our previous finding that the *Drosophila* TBP, which differs from human TBP at the critical position 27 (H→R) in the C1a region, behaves as an intermediate between human and yeast TBPs in EC cells⁶.

We have characterized a signalling pathway *in vivo* that connects an upstream retinoic acid-dependent transactivator, RAR/RXR¹⁸, to the basal transcription machinery by showing that the EC cell-specific E1A-LA must interact with the core domain of TBP for positive regulation of a pol II promoter. We have identified critical residues in the core domain of TBP that map outside but close to the basic region and which define a surface on TBP that is necessary for a functional association with E1A-LA (Fig. 4b, c). Interactions *in vitro* between different gene-specific activators and the core domain of TBP have been reported that involve residues in the basic region of the core domain. These include the lymphoid-specific cellular transcription factor PU.1 (ref. 19), the tumour-suppressor gene product p53 (ref. 20), the adenovirus E1A protein^{8,16} and the herpes simplex virus VP16 (refs 21, 22). That so many proteins bind TBP indicates that the TBP-TAF-coactivator complex must be constantly in flux *in vivo*.

Our study provides insight into the mode of action of the EC cell-specific E1A-LA. In higher organisms many chromographically distinct TFIID complexes can be detected that function in pol II transcription²³. These complexes are thought to be composed of a set of common core TAFs and one or more minor components that function as coactivators or mediators; E1A and E1A-LA may be examples of such components. Many activities have been ascribed to the viral E1A and its presumed cellular homologue, E1A-LA^{6,24-26}. Given the many functions that can be attributed to distinct domains of E1A, it is not unlikely that different cellular factors may fulfil these independent functions. □

The structure of the TBP protein from *Arabidopsis thaliana*¹⁷ has an approximate 2-fold symmetry, with each half consisting of a five-stranded antiparallel β -sheet and two α -helices. The C1a region of human TBP differs by only six amino acids from the same region in the yeast protein (Fig. 3a). Three of these amino-acid substitutions (Q5→T, S11→A and N14→T) map in β -strand 1. K18→R is in the spacer region between β -strand 1 and α -helix 1, which contains the last two substitutions (I24→V and R27→H) (ref. 17 and Fig. 4a). Modelling the human TBP on the basis of the structure of *A. thaliana* TBP¹⁷ indicates that four of these six amino acids are on the convex surface of the TBP molecule and that two residues are buried in the structure (S11→A and I24→V).

Each of the six variant amino acids of the yeast TBP C1a region in the yhh-yC1a/spm3 chimera were reverted to assess which of them could restore the ability to mediate transactivation in conjunction with the E1A-LA. The T14→N reversion in β -strand 1 virtually restored the ability to mediate retinoic acid-dependent transactivation in EC cells, whereas the H27→R in

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PNA hybridizes to complementary oligonucleotides obeying the Watson–Crick hydrogen-bonding rules

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DNA ANALOGUES are currently being intensely investigated owing to their potential as gene-targeted drugs^{1–3}. Furthermore, their properties and interaction with DNA and RNA could provide a better understanding of the structural features of natural DNA that determine its unique chemical, biological and genetic properties^{3,4}. We recently designed a DNA analogue, PNA, in which the backbone is structurally homomorphous with the deoxyribose backbone and consists of *N*-(2-aminoethyl)glycine units to which the nucleobases are attached^{5–9}. We showed that PNA oligomers containing solely thymine and cytosine can hybridize to complementary oligonucleotides, presumably by forming Watson–Crick–Hoogsteen (PNA)₂–DNA triplexes, which are much more stable than the corresponding DNA–DNA duplexes^{5–7}, and bind to double-stranded DNA by strand displacement^{5,8}. We report here that PNA containing all four natural nucleobases hybridizes to complementary oligonucleotides obeying the Watson–Crick base-pairing rules, and thus is a true DNA mimic in terms of base-pair recognition.

A non-self-complementary pentadecamer, PNA, was designed with a GTAC sequence in the middle and containing an almost equal number of pyrimidines and purines, but having no more than two purines or pyrimidines juxtaposed. The thermal stability, measured as the melting temperature, T_m , of complexes between the pentadecamer H-TGTACGTCACAACTA-NH₂, and the complementary deoxyoligonucleotide 3'-d(ACATGCAGTGTGAT) (termed antiparallel orientation: amino terminal of the PNA facing the 3'-end of the oligonucleotide), was 69.5°C (Table 1), whereas the T_m of the corresponding PNA–DNA complex in the parallel orientation was 56.1°C. The

T_m for the PNA–RNA complexes was 72.3 and 51.2°C, respectively (Table 1). The orientation preference was further settled by using two decamer PNAs, and hybridizing these to complementary oligonucleotides in both orientations. The antiparallel orientation was preferred in all cases (Table 1). Furthermore, note that virtually identical T_m s were obtained regardless of which strand is the PNA and which is the DNA (Table 1, and columns 2 and 3). The results also show that the presence of the terminal lysine amide, which was contained in our original PNA to reduce aggregation of oligothymine PNA^{5,6}, does not influence the preferred orientation of the PNA relative to the DNA.

When a Watson–Crick base-pair mismatch was introduced in the oligonucleotide at any position facing the four middle PNA nucleobases (GTCA) in the pentadecamer, a large decrease in T_m (8–20°C; Fig. 1) was observed, thereby providing compelling evidence that PNA–DNA recognition takes place by Watson–Crick base pairing, that is, A·T and G·C base pairing. For comparison we also measured the thermal stabilities of the corresponding DNA–DNA duplexes (Fig. 1). Note that for virtually all base-pair mismatches, the decrease in thermal stability is greater for the PNA–DNA complex than for the DNA–DNA complex, suggesting (provided that the differences in transition enthalpies are identical) that the sequence discrimination is, if anything, more efficient for PNA recognizing DNA than for DNA recognizing DNA.

The unambiguous evidence for Watson–Crick base pairing suggests that these PNA–DNA complexes are duplexes rather than the (PNA)₂–DNA triplexes previously observed with homopyrimidine PNA^{6,7}. This conclusion was confirmed by titration experiments using ³²P-labelled oligonucleotides in a gel retardation assay (Fig. 2a) or using circular dichroism (CD) for the detection of complex formation (data not shown).

FIG. 1. Thermal stabilities of PNA–DNA complexes. *a*, Chemical structure of PNA and DNA. B is the nucleobase, and R¹ = H, or lysinyl amide for the PNAs discussed here. The PNAs are written from the N to the C terminal using normal peptide conventions: H, a free amino group; -NH₂, a terminal carboxamide. The PNAs were synthesized as previously described^{6–8}. They were purified by HPLC and their composition was verified by mass spectrometry. *b*, Effect of base-pair mismatches on the thermal stability of PNA–DNA complexes. The thermal stability of complexes between PNA, H-TGTACGTCACAACTA-NH₂ and the 13 oligonucleotides 3'-d(ACATGXZYWGTTGAT), in which X, Y, Z, W = C, A, G, T for the case in which the PNA and DNA sequences are complementary. In each of the 12 other oligonucleotides, three of the bases (X, Y, Z, W) were complementary and the fourth was one of the three non-complementary nucleobases. For example, when X = T, Y = G, Z = A, then W = A or C or G and so on. Thus each of the 12 oligonucleotides contains one of the 12 possible base-pair mismatches relative to the PNA pentadecamer. The T_m s of these complexes are displayed as solid bars. For comparison, the results of similar experiments with DNA–DNA duplexes are shown (hatched bars). *c*, Effect of ionic strength on the thermal stability of PNA–DNA (○) or DNA–DNA (●) complexes.

METHODS. Hybridizations were done in 10 mM sodium phosphate, pH 7.0, 150 mM NaCl, 1 mM MgCl₂. Qualitatively similar results were obtained using oligonucleotides in the parallel orientation.

¶ To whom correspondence should be addressed.

Information about secondary structure may be obtained from CD measurements because these are sensitive to the base-pair geometry in the helix. Figure 2b shows the CD spectra of the PNA-DNA, PNA-RNA, DNA-DNA and DNA-RNA duplexes. The CD spectra of DNA-DNA and antiparallel PNA-DNA and PNA-RNA duplexes are largely similar, suggesting that these PNA-DNA and PNA-RNA duplexes are right-handed helices with a base-pair geometry not much different from that found in a B- or an A-form DNA helix. But it is interesting that the CD spectra, and thus the structure, of the parallel versus antiparallel PNA-DNA (or PNA-RNA) duplexes are distinctly different.

Thermodynamic parameters for hybridization were extracted from thermal stability measurements^{10,11} to determine ΔH^0 , ΔS^0 and ΔG^0 for formation of the PNA-RNA, DNA-RNA, PNA-DNA and DNA-DNA duplexes (Table 1). It is remarkable that the decrease in entropy is almost identical for the formation of DNA-DNA and PNA-DNA duplexes. This implies that the single-stranded PNA has the same degree of organization as single-stranded DNA.

The kinetics of PNA-RNA duplex formation were also measured, and the results show that the rate of hybridization ($\sim 10^6 \text{ M}^{-1} \text{ s}^{-1}$, data not shown) is at least as fast as that of DNA-DNA duplex formation. Again, this is fully consistent with the suggestion that the single-stranded PNA is at least as prestructured for duplex formation as is DNA¹².

The molecular forces that are responsible for the unique chemical and biological properties of DNA are still not fully understood. In particular, it is not clear to what extent the Watson-Crick base-pair recognition is an intrinsic property of the nucleobases themselves or how much is due to the rigid structural framework provided by the deoxyribose phosphate backbone. For instance, homo-DNA having hexoses in the backbone does not display Watson-Crick base pairing⁴. Our finding that an acyclic backbone, such as that of PNA, confers DNA-mimicking hybridization properties argues for a key role of nucleobase stacking. Of course we cannot rule out that fortuitous intramolecular interactions of the backbone direct this DNA-like geometry. But it is unlikely that such interactions in PNA-DNA hybrids would be favourable regardless of the orientation of the PNA relative to the DNA; stable PNA-DNA hybrids are formed in both the parallel and antiparallel orientation (Table 1).

TABLE 1 Melting temperatures T_m ($^{\circ}\text{C}$) for PNA-DNA, PNA-RNA, DNA-DNA and DNA-RNA complexes

First-strand sequence*	TGTACGTCACAATA†	GTAGATCACT‡	AGTGATCTAC‡
PNA:DNA (parallel)	56.1	38.0	38.0
PNA:DNA (antiparallel)	69.5	51.0	49.0
PNA:RNA (parallel)	51.2	ND	ND
PNA:RNA (antiparallel)	72.3	ND	ND
DNA:DNA	53.3	33.5	33.5
DNA:RNA	50.6	ND	ND

Absorbance versus temperature curves were measured at 260 nm in 100 mM NaCl, 10 mM sodium phosphate, 0.1 mM EDTA, pH 7, as described in ref. 11. T_m , the temperature at which half of the molecules are hybridized, was obtained by fitting triplicate melting curves at 4 μM of each strand to a modified two-state model with linear sloping baseline²⁰.

* Written 5'-3' for oligonucleotides and N to C terminal for PNA.

† The PNA terminates in a carboxamide.

‡ The PNA terminates in a lysine amide.

The PNA backbone is uncharged, and we ascribe the increased thermal stability of the PNA-DNA duplex relative to that of the DNA-DNA duplex predominantly to lack of electrostatic repulsion between the two strands. This contention is supported by experiments showing that PNA-DNA and DNA-DNA duplexes have equal thermal stability at ionic strength above 1 M Na^+ (Fig. 1c).

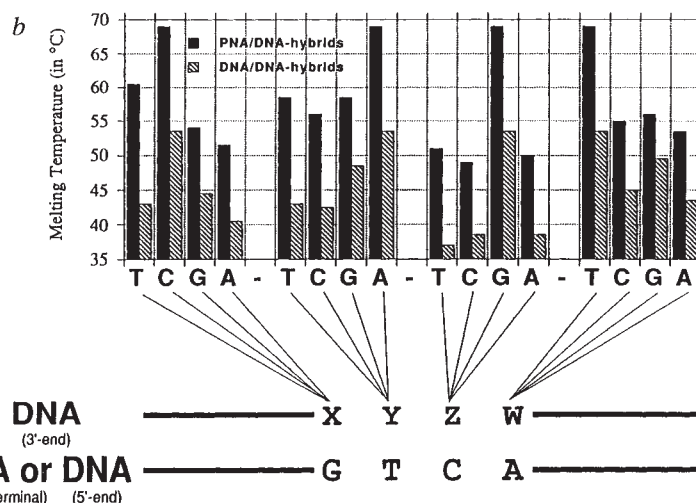
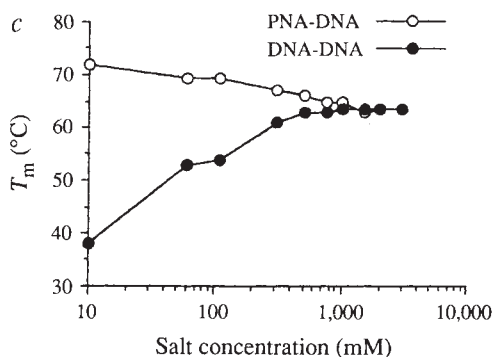
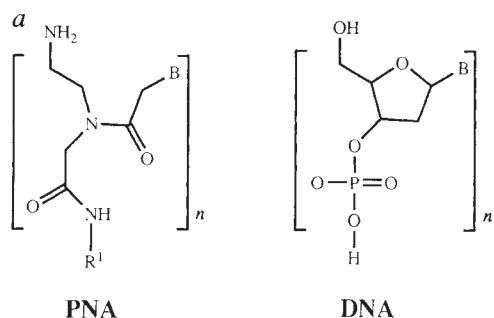
These results may also throw light on the evolution of genetic material. It is now commonly argued that our present-day DNA

TABLE 2 Thermodynamic parameters for the formation of PNA-DNA, PNA-RNA, DNA-RNA and DNA-DNA duplexes with the sequence TGTACGTCACAATA present in the PNA strand strand

	DNA:RNA	PNA:RNA	DNA:DNA	PNA:DNA
ΔH^0 (kcal mol ⁻¹)*	-128.9	-128.5	-105.3	-106.6
ΔS^0 (EU)*	-372.8	-345.9	-296.2	-285.8
ΔG^0_{37} (kcal mol ⁻¹)*	-13.3	-21.2	-13.4	-18.0
T_m ($^{\circ}\text{C}$, 8 μM)*	50.1	72.2	53.5	68.8

Measured in 100 mM NaCl, 10 mM sodium phosphate, 0.1 mM EDTA, pH 7.0.

* Obtained from linear plots of $1/T_m$ versus $\log(\text{concentration})$ ²⁰.



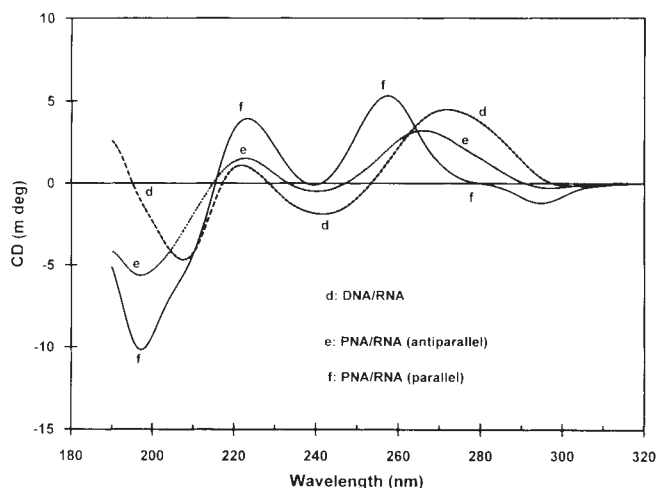
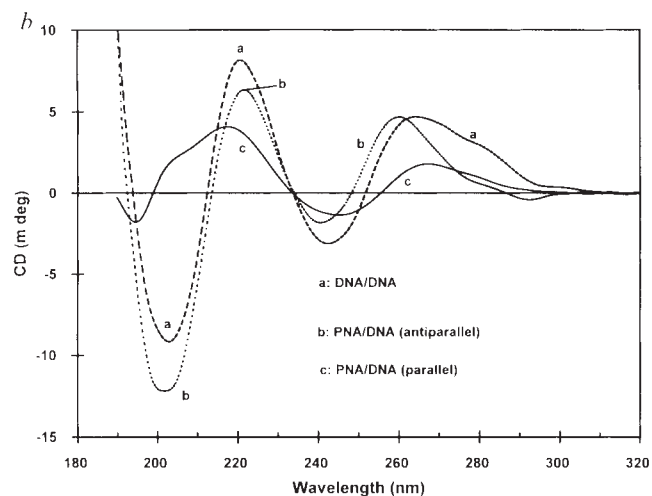
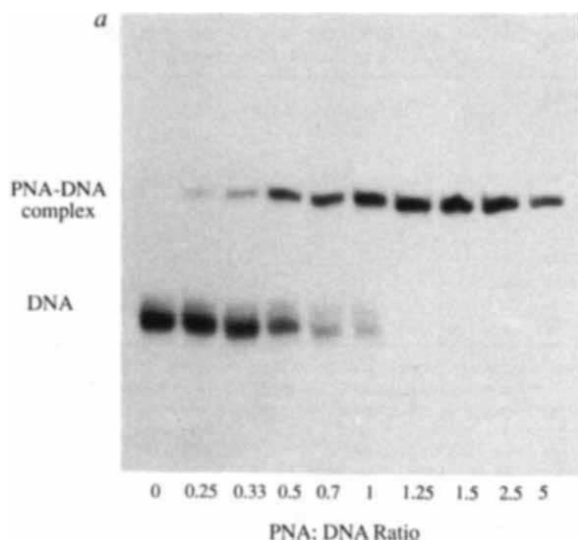


FIG. 2 Structural properties of PNA-DNA complexes. *a*, Titration by gel-shift of the binding of PNA H-TGTACGTCACA-CTA-NH₂ to the 5'-end labelled oligonucleotide 3'-d(ACATGCAGTGTGAT). *b*, Circular dichroism spectra of PNA-DNA (*b*, antiparallel; *c*, parallel), PNA-RNA (*e*, antiparallel; *f*, parallel), DNA-DNA (*a*) and DNA-RNA (*d*) complexes. The DNA sequence (*a*-*c*) was 3'-d(ACATGCAGTGTGAT), and the RNA sequence (*d*-*f*) was 3'-ACAUGCAGUGUUGAU.

METHODS. The oligonucleotide was labelled with ³²P at the 5' end using standard techniques²¹. The oligonucleotide (1 nmol; 10³ c.p.m.) was incubated with various amounts of PNA (0-5 nmol) in 10 μl 10 mM Tris HCl, 1 mM EDTA, pH 7.4, for 1 h at 37 °C. The samples were analysed by electrophoresis in 20% polyacrylamide gels (TBE buffer, 89 mM Tris-borate, pH 8.3, 1 mM EDTA) and the radiolabelled DNA visualized by autoradiography. Concentrations of oligonucleotides and PNA were measured photometrically. Similar results were obtained using the complementary oligonucleotide of reversed polarity (5'-d(ACATGCAGTGTGAT)). Complexes for circular dichroism were formed by mixing equal molar amounts of the two complements in distilled H₂O. Circular dichroism spectra were recorded on a Jasco 700 instrument at room temperature using an optical path of 1 mm. All measurements were averaged 10 times and smoothed.

world was preceded by an RNA world^{13,14}. But RNA is a chemically fragile molecule, unlikely to survive the harsh prebiotic conditions. Note that a 'peptide nucleic acid' like PNA has the recognition properties of DNA and consequently the potential to carry genetic information. It has been shown that both amino acids¹⁵ and nucleobases¹⁶ are formed under conditions designed to mimic the 'prebiotic soup'. Thus it is conceivable that 'peptide nucleic acid'-like compounds could also have been formed, and might have played a prebiotic role. Finally we note that the properties of PNA reported here, especially their capacity for sequence recognition and hybrid stability, emphasize the potential of such compounds as antisense drugs¹⁷⁻¹⁹. □

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ERRATUM

Lethal effect of the anti-Fas antibody in mice

Jun Ogasawara, Rie Watanabe-Fukunaga, Masashi Adachi, Akio Matsuzawa, Tsutomu Kasugai, Yukihiko Kitamura, Naoto Itoh, Takashi Suda & Shigekazu Nagata

Nature **364**, 806-809 (1993)

DURING the production process, a line of text in this letter was accidentally omitted. The fourth sentence of the last paragraph should read, "The liver showed gross injury by administration of the anti-Fas antibody and acute hepatic failure seems to be responsible for the death of mice, although we cannot rule out lethal damage in tissues such as heart and lung." □

New Journals review 1993

CRITERIA for journals to be considered for review in this issue were circulated to publishers earlier this year, and were also published in *Nature*. They were that:

- (1) the first number appeared during or after June 1991 and at least four separate numbers were issued by the end of April 1993 (although some of the journals eligible for, but not covered in, last year's review issue were also considered)*;
- (2) the journal is published at least three times a year;
- (3) the main language used is English;
- (4) where possible, at least four issues should be made available for review, including the first and the most recent numbers.

The time criteria ensure that a reasonable sample of issues is available for judgement by the time reviews are commissioned.

Several journals known to satisfy the criteria were not submitted for review, or arrived too late for inclusion. It proved difficult to find reviewers for other, doubtless worthy journals, while some titles were considered to be of marginal interest to *Nature's* audience. Journals covering any aspect of science were eligible, although those dealing with clinical medicine, engineering and pure mathematics were excluded, as were abstracts publications and newsletters. A list of titles eligible for review but not covered appears on page 589.

The brief given to the reviewers was to limit themselves to comments on the publications sent to them, and to avoid discussion of general questions of periodical publishing. Opinions expressed in the reviews are based on a sample of issues, and apply to mid-1993 at the latest. As in previous years, the preponderance of journals in the biological sciences reflects the bias of material submitted.

Details of editors and frequency of publication, and the subscription rates appearing at the top of each review, are given in most instances for 1994. This information is not complete in all cases, and readers interested in subscribing to a particular journal should check the rate with the publisher concerned. □

* See *Nature* 359, 435–464 (1992); 353, 457–481 (1991); 347, 581–599 (1990); 341, 350–370 (1989); 335, 459–478 (1988); 329, 357–376 (1987); 323, 359–379 (1986); 317, 293–308 (1985); 311, 309–330 (1984); and 305, 477–497 (1983).

Triplet expansions

Bert Vogelstein

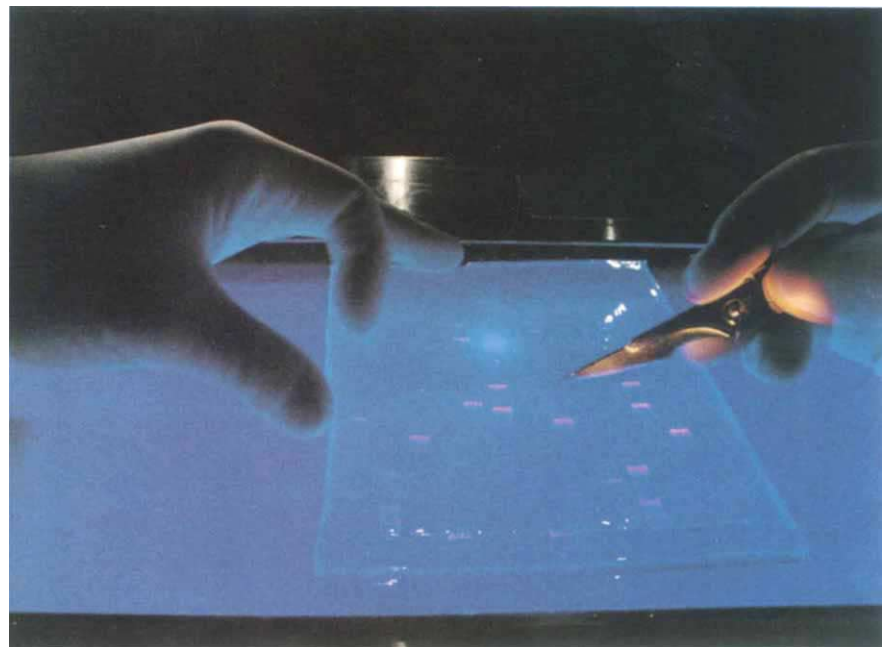
Nature Genetics. Editor Kevin Davies. *Nature Publishing*, New York. 12/yr. USA and Canada \$495, UK £350 (institutional); USA and Canada \$195, UK £175 (personal).

Human Molecular Genetics. Editors Kay E. Davies and Huntington F. Willard. IRL/Oxford University Press. 12/yr. Europe £250, elsewhere \$435 (institutional); Europe £80, elsewhere \$140 (personal).

Human Mutation. Editors R. G. H. Cotton and Haig H. Kazazian Jr. Wiley. 8/yr. USA \$250, Canada and Mexico \$330, elsewhere \$360 (institutional); USA, Canada and Mexico \$125, elsewhere \$165 (personal).

As an avid and somewhat compulsive reader of the literature, I generally wince at the publication of a new journal — yet more pages to turn, more data to imbibe. But in some fields, new technological developments make new journals imperative, simply because the volume of data becomes too great for established journals to handle. Such is clearly the case in

sponsible for various conditions, *Nature Genetics* also provides a substantial amount of biological and conceptual information about genetic disease. Its papers seem to be carefully selected, especially in recent issues, and a considerable proportion of them present seminal, rather than follow-up, studies. Particularly appealing has been the



Peter Ginter

The cutting edge — separation of protein by gel electrophoresis. This award-winning photograph appears in *World Press Photo 1993* (Thames and Hudson, £8.95).

genetics, human genetics in particular, where the number of mutations discovered to cause disease is rising exponentially. Part of this rise can be traced to an exponential technique — the polymerase chain reaction, which makes it possible to identify mutations with a speed that was unimaginable even a few years ago. Because these mutations provide new insights into the pathogenesis of various diseases, papers describing them have become instrumental in several fields, most notably the study of hereditary diseases and cancer.

A triplet of new journals has been born on this basis. *Nature Genetics*, a spin-off of *Nature*, is a superb entry. Although, as expected, it describes the mutations re-

thematic grouping, with two or four papers on a similar topic clustered in a single issue. Past issues have thus included clusters presenting ground-breaking studies on Menkes, Charcot-Marie-Tooth and Alzheimer's diseases. Similarly, several issues have contained collections of papers on the triple-repeat expansions characteristic of fragile X syndrome, myotonic dystrophy and Huntington's disease. The papers on triplet repeats cover technical, biological, clinical and genetic aspects, and are required reading for anyone interested in this burgeoning topic. Most articles are of nearly ideal length — short enough to be carefully read, long enough to contain adequate experimental detail. The journal's accom-

panying commentaries are in 'News and Views' style and are in general scholarly, with a dash of humour, clearly rivalling those of its parent. As a result of all this, *Nature Genetics* is one of a handful of journals whose monthly arrival I now eagerly await.

Human Molecular Genetics is a spin-off of *Nucleic Acids Research*. Both it and the third new journal, *Human Mutation*, have distinguished, international editorial boards. In both cases, the editors have accomplished what they set out to do: to provide a reputable journal devoted to publishing papers describing the genetic analysis of human hereditary diseases. The journals are true to their names, and *Human Molecular Genetics* thereby has a wider scope, with most of its articles dealing with basic studies of gene expression, physical mapping and linkage related to human genes of interest. The quality and content of these articles are in general what one would expect from a top-notch specialty journal. *Human Molecular Genetics* has so far attracted a more interesting and varied collection of papers than *Human Mutation*. Almost all the papers in *Human Mutation*, and about a quarter of those in *Human Molecular Genetics*, report additional examples of mutations in diseases already described at the genetic level. Although a bit archival (the molecular equivalent of clinical case reports), such papers can be useful to

investigators studying the disease and have obvious clinical importance. Documentation of the mutations is usually robust and a subset of the papers reports useful technical modifications that have aided the identification of mutations and that occasionally have general applicability. Each of these two journals has a special feature. Nearly every issue of *Human Mutation* carries a brief review of mutations in a specific gene; these reviews are well written, concise and up to date. *Human Molecular Genetics* has a section devoted to the description of new, highly polymorphic markers, and currently provides the best vehicle for publishing (and finding) such polymorphisms in the literature.

In summary, each of the three journals provides authors and readers with a valuable resource for a variety of studies relevant to human genetic disease. *Human Molecular Genetics* and *Human Mutation* deserve to be in every library catering to a community of human geneticists. *Nature Genetics* has broader appeal, lying squarely at the interface between molecular biology, genetics and medicine; many will find a personal subscription rewarding. □

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The acceptable face of science?

Walter Gratzer

Helix: Amgen's Magazine of Biotechnology. Editor James Geary. 3/yr. Bugamor International, Haak 58, 1353 AE Almere-Haven, The Netherlands. Distribution details available on application to publisher.

HERE is a good deed in a naughty world, a new journal that no one can deplore, a source for once of pleasure and not of pain. Imagine having to think up a name for a new biotechnology company — the more euphonious variants on the Biogen, Genentech, Celltech theme, even including (in Italy) Genitalia, being surely now more or less exhausted — let alone a new journal. *Cell* and *Gene* have gone, not to mention *Brain*, *Blood*, *Gut* and *Bone*. The surface scientists had an imaginative breakthrough with *Langmuir*, so perhaps we may soon see volume 1 of *Crick*, *Watson* or *Pauling* (Linus even?) hit the library shelves.

Well, Amgen (a leader in the biotechnology stakes) has come up with *Helix*, surprisingly hitherto overlooked in the scramble for numinous titles, and a felicitous choice. For the helix is surely a potent symbol for our time. Salvador Dali, who represented one in a painting, believed that he had prefigured the discovery of the Thread of Life. The mediaeval divine, Origen, taught that since the sphere was the most perfect of all objects, all points on its surface being equidistant from the centre, the virtuous among us would, come the Day of Judgement, be transformed into spheres and enter paradise rolling. Perhaps, then, a trendier theology will have us changed into helices — so much richer in symmetry and imagery — to enter paradise screwing.

Only in the third issue of *Helix* is there actually a dissertation on the helix and its place in nature and in architecture, agreeably written by John Galloway and captivatingly illustrated. The graphics and the photographs, many of them light- and electron-microscope images in false colour, are superlative throughout and the production of *Helix* has an aesthetic panache that makes it a rare pleasure to turn the pages. There is clearly here a vision of science as art and I hope the journal will reach a wider public than the biological community alone. The name of Amgen appears in small print on the cover and there is nothing in the text in any of the issues that so much as hints at promotion. The scientific articles reach out to the nonspecialist and are at about the level of *Scientific American*. The best are excel-

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lent; H. Schellekens on mitochondrial DNA and P. K. Vogt on oncogenes are two examples that I found crisp and illuminating. There are also interviews with important personages — Nobel laureates, journal editors and the like. This is a difficult genre, and here (at least to my mind) only moderately successful, for there are too few surprising revelations and no outrageous opinions are ventilated. Book reviews too suffer from a certain decorum (does Gallo have to be treated with quite such servility?). A few errors have slipped through undetected: Chaim Weizmann, for instance, should not have been allowed to pass as a colleague of Pasteur's.

But it is ungrateful to cavil where there is so much to enjoy. There are rewarding pieces by experts on such topics as the great medical illustrators, on alchemy, Taoist physiology, Vesalius, Willem de Kooning and Frank Lloyd Wright. The distinguished author of the last — not a scientist — does not remark on the helical fine-structure of the Guggenheim Museum in New York, prominently on view in two sumptuous photographs. Wright, incidentally, also had something to say on clinical science: doctors, he observed, can always bury their mistakes, but an architect can only advise his clients to plant vines.

It remains only to ask Amgen how they will select the lucky recipients of their covetable confection. Beg a subscription if you can! □

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Powerful knowledge

Susan Michie

Journal of Genetic Counseling. Editor-in-chief Deborah L. Eunpu. *Human Sciences*. 4/yr. USA \$95, elsewhere \$100 (institutional); USA \$40, elsewhere \$45 (personal).

As the work of the Human Genome Project continues, technology is being rapidly developed for an increasing range of screening and diagnostic tests for genetic diseases and conditions in individuals and their families, present and future.

Many questions are raised. How should the tests be presented? How should the uncertainty of risk be conveyed? How can the distress associated with 'positive results' be minimized? How are genetics and inheritance best explained? What is an 'informed decision'? What should family members be told?

There is a great need for such questions to be studied empirically and for results to be communicated to those providing, and purchasing, genetics services. The appearance last year of *Journal of Genetic Counseling* is therefore to be welcomed. This publication aims to provide genetic counsellors and other genetics advisers with "careful examination of the methods used to convey genetic information". It does so by peer-reviewed research, essays, review articles and letters.

A variety of issues are covered, such as the role of genetic counsellors in different services, cultural barriers to genetic ser-

vices and how to overcome them, and practical recommendations for counselling. So far, issues have contained papers that tend to discuss questions rather than provide data for addressing them. The all-American editorial board perhaps accounts for the lack of contributions from outside the United States. But the journal is a promising beginning in an area with great potential. □

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In the footsteps of Pasteur

F. E. G. Cox

Immunology and Infectious Diseases. Editor R. K. Chandra. *Rapid Communications of Oxford*. 4/yr. £235, \$395 (institutional); £82, \$140 (personal).

Vaccine Research. Editor Michael G. Hanna Jr. Liebert. 4/yr. USA and Canada \$149, elsewhere \$189.

Infectious Agents and Disease. Editor-in-chief Bernard Roizman. *Raven*. 6/yr. USA and Canada \$138, elsewhere \$171 (institutional); USA and Canada \$98, elsewhere \$119 (personal).

THERE is obviously a lot of immunological research being done and lots of journals in which the results can be published, so any new venture must fill an important and distinct niche; otherwise it merely becomes a dumping ground for the ephemera of the subject.

The present massive field of immunology originally grew out of studies on immunity to infection but, apart from *Immunity and Infection* and *Vaccine*, there are few journals dedicated to this area. So there does seem to be a niche for one or more new publications. *Immunology and Infectious Diseases* starts off with a very wide brief to publish original articles, clinical trials, brief communications, critical reviews and hypotheses on topics such as immunoregulation, immunocompetent cells, cytokines, cancer and autoimmune disease; in other words, much of the same as one might find in any one of a hundred publications. In fact, the range of the 50 or so articles published each year is immense, but few, if any, seem to be in the front line of immunological research. What is new, however, is the aim to publish papers within 12 weeks of acceptance, an ambition achieved a few times in the first issue but seldom since, with the publication time drifting more recently to an average of 10 months, and in one case 18 months. In summary, this is just another general immunological journal.

Vaccine Research "provides a central forum of documentation of basic and applied research in vaccine development and application" and publishes reviews, articles, reports, brief communications, letters and leading articles. Vaccines are becoming increasingly important and a journal such as this could be a welcome addition to the literature, especially if



Point of departure — vaccination against cholera. From *La Science Illustrée* (c. 1890).

it covers veterinary topics. There are some good reviews but, in the main, the contents consist of five or six fairly ordinary papers mainly on experimental aspects of immunogenicity. Part 3 of volume 1 is devoted to the proceedings of a meeting on mucosal immunity and AIDS, and contains very little about vaccines. One doesn't get much for one's money because of the lavish use of space: 20 references occupy a whole page, for example. There is little evidence so far that *Vaccine Research* is going to be anything more than of peripheral interest to those working in the mainstream of this subject, although the occasional review might be well worth reading.

Two down and one to go. *Infectious Agents and Disease*, subtitled *Reviews Issues and Commentary*, publishes

mainly invited reviews, mini-reviews, commentaries and issues of the day. The topics published so far have been very wide ranging, from the application of crystallography in the design of viral agents to the pathogenicity of *Entamoeba histolytica* and cytokines in helminth infections. Although the authors are all acknowledged experts, their contributions vary considerably in clarity and depth. The obvious comparison must be with *Immunology Today*, which is much better value for money, with 516 pages in 1992 (compared with 342) and more than twice as many articles. On the other hand, *Infectious Agents and Disease* is concerned with the real world of immunity to infection and is an attractive and authoritative publication that will appeal to many immunologists, microbiologists, parasitologists and clinicians. Of the three journals, *Infectious Agents and Disease* is the one most likely to succeed. □

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Muscling in

Malcolm J. Jackson

Basic and Applied Myology. Editor-in-chief U. Carraro. Unipress, Padova, Italy. 4/yr. Italy IL150,000, elsewhere \$150 (institutional); Italy IL100,000, elsewhere \$100 (personal).

Neuromuscular Disorders. Editor-in-chief V. Dubowitz. Pergamon. 6/yr. £217, \$335 (institutional); £80, \$123 (personal).

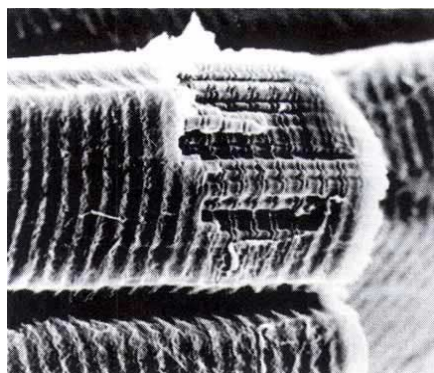
THE first leading article in *Basic and Applied Myology* (BAM) asks whether the new professional activity of 'myology' is emerging. From the clinical point of view, skeletal muscle has usually been placed in the field of neurology with papers mainly being published in neurological journals. The number of specialist journals for muscle researchers has been small. These two new journals are both seeking to attract papers from the group whose prime area of interest is muscle. Both are interdisciplinary: *Neuromuscular Disorders* covers both clinical and basic sciences of relevance to the neuromuscular diseases of childhood and adult life, whereas BAM aims to cover basic skeletal-muscle research and its clinical applications.

Muscle contraction has kept traditional physiologists busy for many years, but the past decade has seen many other disciplines looking at this tissue: molecular biologists have played an important role in the elucidation of the underlying defects

in Duchenne, Becker and myotonic muscular dystrophies, whereas cell biologists have become fascinated by the techniques and prospects of cell therapy for muscle disorders; even electronic engineers have been involved, developing implantable stimulators for electrical stimulation and transforming skeletal muscle fibre types for cardiac repair.

Both journals attempt to provide a forum for the publication of papers from many different disciplines. Overall there have been more papers on basic science in BAM and, among the original articles and reviews, a high proportion have dealt with the use of skeletal muscle in the assistance of failing cardiac tissue. BAM is well presented, with good-quality electron micrographs and histology plates.

Neuromuscular Disorders is also well presented. It is more clinically oriented, and publishes a wide range of papers from



Scanning electron micrograph of human striated muscle fibres ($\times \sim 1,150$).

case reports to basic science. The reviews are excellent, particularly those related to recent advances in the cell and molecular biology of the muscular dystrophies. The journal seeks to provide a service to workers on genetic aspects by publishing a regularly revised table of gene locations and associated data for neuromuscular disorders. There are also lists of relevant, recently published papers, book reviews and conference reports.

Whether the muscle field can sustain two new journals remains to be seen. There is little direct competition: only *Muscle and Nerve* and perhaps *Journal of Muscle Research and Cell Motility* compete for a similar market. Initial submission rates look promising for *Neuromuscular Disorders*, which has had a rejection rate of about 50 per cent over its first two years. A more serious threat to the survival of both of these new publications will be the increasing financial constraints on institutional subscriptions. I hope the journals succeed. □

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Bridging gaps

Jean Merrill

Advances in Neuroimmunology. Editors G. B. Stefano and E. M. Smith. Pergamon. 4/yr. £189, \$290 (institutional); £76, \$117 (personal).

ADVANCES IN NEUROIMMUNOLOGY was launched and is still manned by several pioneers in this fast-evolving interdisciplinary field. Its goal is to publish short, up-to-date reviews on the two-way communication between the nervous and immune systems, in particular its relation to evolution, development, homeostasis and pathology. The journal's competitors are few, if any; no other journal exclusively promotes reviews in this field and only one or two journals are dedicated to publishing original scientific articles on the biological links between the two systems. The recent trend among some neuroscience journals to feature special issues that specifically address neuroimmune interactions may change this unique standing of *Advances*. But there is clearly a need for such a review journal. Although *Advances in Neuroimmunology* has some growing to do, it shows great promise.

The nature of the articles has varied over the years, with some issues stressing neuroimmune themes that might otherwise have been buried in speciality journals. An example of this is the interesting issue on the evolution of neuroimmune interactions. The review articles are important not only for bringing the reader up to date; many also have an exploratory and speculative component that serves a dual purpose of bridging the gap between studies in neuroscience and immunology and fostering creative new ideas that can be addressed in the laboratory. Recent issues on highly focused areas (neuro-AIDS, cytokines) have been guest-edited, breathing freshness into the contents.

Since its inception, the journal has changed shape, becoming smaller in size (trimmer in width and down to only five articles per issue). It is printed on glossy high-quality paper. Photographic illustrations, however, are few in number and mostly black and white. The trade-off is that there are no page charges to authors, and articles are published fairly rapidly. The subscription rate is a bit pricey for only four issues and prompts one to wish for more in terms of both quality and quantity. Although the luxury of subscribing to this journal may be unattainable for the individual, neuroscience and immunology departmental libraries should have copies on their shelves. □

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Bitter pills?

John Hughes

Pharmacology Communications. Editors-in-chief N. G. Bowery and R. R. Ruffolo Jr. *Harwood Academic*. 4/yr. \$242 (institutional); \$95, £63 (personal).

Pharmaceutical and Pharmacological Letters. Editors P. Dominiak et al. *Springer*. 6/yr. DM360, \$225.50.

In a world of sound bites and photo opportunities there is a definite attraction for a scientist in being able to publish short papers rapidly in a glossy printed format. Both of these new journals have the similar and rather ambitious aims of covering all of pharmacology and therapeutic research. The editors-in-chief of *Pharmacology Communications* lament the trend towards specialist journals and hope their journal will reverse the fashion, a forlorn hope I fear, because most scientists have neither the time nor inclination to wade through the pot-pourri of articles appearing in these journals.

Once upon a time, pharmacology was an appendage of pharmaceutical research, but now professional pharmacologists practising in what is a mainstream subject are hardly likely to be enthusiastic about encapsulation technology or the composition of excipients, however worthy the research. In truth, the market for general interest journals was long ago captured by *Nature*, *Science* and the excellent Elsevier *Trends* series for reviews. Scientists will read outside their area if the material is sufficiently compelling and authoritative, but this is true for only a small core of general interest journals.

What then might these journals achieve in the way of rapid publication of articles containing sound but limited data? *Pharmacology Communications* is on stronger ground here because virtually everyone has experienced the pain of rejection couched in the general terms of "worthy but requires further experiments/analysis/alternative approaches. . .". Most journals of pharmacology offer rapid publication of short articles of immediate interest and I suspect that these two journals will find it difficult to compete in this area. The problem is that although most of the articles in these journals are of reasonable scientific quality, one is left feeling undernourished — too much bread and not enough meat. There is a fallacy, of course, in the assumption that length is somehow related to the scientific acceptability of a particular study. *Pharmacology Communications* recommends that articles should not exceed 2,000 words, with no apparent limit on figures or tables, whereas *Pharmaceutical and Pharmacological Letters* enjoins authors not to exceed four pages of roughly 1,300 words a page.

There are significant differences between these two journals. *Pharmacology Communications* has an impressive international board of associate editors and editors, and papers can be submitted to the editors-in-chief or to associate editors. Articles can be submitted on disk and the journal format is bright and glossy and the instructions to authors are clear and concise. *Pharmaceutical and Pharmacological Letters* has a small but distinguished board of editors, and papers in camera-ready format should be submitted to the managing editor. The camera-ready format gives an uneven look to the journal.

The contributors to these journals highlight their differences. *Pharmaceutical and Pharmacological Letters* is almost entirely composed of articles from academic departments and institutes with a strong bias towards pharmaceuticals and medicinal chemistry. About 40 per cent of the articles in *Pharmacology Communications* are from the pharmaceutical industry with one company, not unrelated to one of the editors-in-chief, supplying the bulk of these articles. The journals will fulfil a need simply because there is a seemingly inexhaustible number of authors seeking a home for their scientific orphans, but I doubt that the publications are destined to become important source journals in pharmacology or pharmaceuticals. □

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Seeing the light

Richard J. Cherry

Journal of Fluorescence Editor-in-chief Joseph R. Lakowicz. *Plenum*. 4/yr. USA and Canada \$175, elsewhere \$205 (institutional); USA and Canada \$40, elsewhere \$47 (personal).

FLUORESCENCE long ago escaped from the laboratories of physicists and chemists into the hands of biochemists and cell biologists, ever eager to find new laboratory tools. The apparently ever-growing application of fluorescence in the biological sciences is evident in the range of fluorophores offered by the leading supplier. Although overshadowed in quantity by biological applications, there is also still a large interest in fluorescence in the fields of chemistry and photophysics as well as optical sensing.

Unlike other spectroscopic techniques, such as magnetic resonance, there has until now been no specialist journal for fluorescence. *Journal of Fluorescence* thus clearly fills a gap. The stated scope of the new journal is something of a catch-all,

but the emphasis is clearly on advances in technique, theory and data analysis. It is in these areas that it is particularly appropriate to provide a focus for papers that have previously been dispersed in the literature. In the early issues, about a half of the contributions fall into these categories, whereas the ratio of biological to chemical applications is about 3 to 1.

Journal of Fluorescence has a distinguished editorial board; several members have contributed to the early issues and have set a good standard. The quality of production is pleasing, and the journal is reasonably good value for money. Given that the pace of innovation in fluorescence shows no sign of diminishing, it has a good chance of success. □

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Signals galore

Michael J. Berridge

Biological Signals. Editors-in-chief S. F. Pang, T. Fujita and P. A. Ward. *Karger*. 6/yr. SFr75, \$250 (institutional); SFr187.50, \$125 (personal).

THE Institute for Scientific Information has identified cell signalling as one of the largest research fields in modern biology. With so much activity going on, there have been rich pickings for scientific publishers ever ready to exploit any new opportunity. A cursory glance through *Current Contents* reveals a plethora of journals covering all aspects of signalling. Even though Karger has already spawned such titles as *Neuroendocrinology* and *Hormone Research*, it has now produced another addition to the family. It is not immediately apparent whether the new member is necessary: it seems to cover much the same area as its siblings.

The intention is for *Biological Signals* to be the repository for papers on the "production, transmission, recognition, processing, modification, and effect of biological signals". This is certainly an ambitious undertaking because it covers the whole field of cell signalling. Many other journals in this field have adopted the opposite strategy of publishing papers on specific signals, such as calcium (*Cell Calcium*), second messengers (*Second Messengers and Phosphoproteins*), steroids (*Steroids*), amines (*Biogenic amines*) and arachidonic acid metabolites (*Prostaglandins*). By opting for a much more general coverage, *Biological Signals* will be able to adapt to new trends and this may be one of its strengths. Its

future will depend on how well it can compete not only with these specialist journals but also with its many rivals (such as *Cellular Signalling*, *J. Neuroendocrinology*, *Endocrinology*, *Acta Endocrinologica*, *J. Endocrinology*, *Molecular Endocrinology*, *Molecular and Cellular Endocrinology*, *Endocrine Research*, *J. Receptor Research*, *Biofactors*, *Neuropeptides and Regulatory Peptides*) which also attempt to cover signalling in a more general way. It is amazing just how ingenious publishers have been in finding titles for journals on cell signalling.

In addition to those already mentioned, *Biological Signals* will be competing with its stable companions *Neuroendocrinology* and *Hormone Research*. In fact, at least half of the 35 papers appearing in the first volume would have been equally at home in *Neuroendocrinology*. These papers deal mainly with the characterization of hormones and their receptors. Some of the other papers are concerned with various transduction processes (the action of G proteins and the release of stored calcium), and the signals responsible for stimulating cell growth are also represented. So far, however, the content is heavily biased towards classical endocrinology.

The editors have put together an impressive editorial board drawn from many disciplines, thus reflecting the wide scope of the journal. As might be expected for a new journal, the board members have contributed more than half of the papers in the first volume. Although it is hoped that they will continue to support this new venture, the success of *Biological Signals* will depend on its ability to attract good papers from outside contributors. So far, the papers are of a reasonably high standard and are beautifully produced. The type is large, making it easy to read, and the halftones give the journal a pleasing uniformity by being set against a stippled background. Unfortunately, the much hated numbering system, which lists references in order of their appearance, makes it difficult to identify whether specific references are present.

Most librarians, however, may think twice about ordering *Biological Signals*, especially since the contents of this somewhat slim journal may well be covered by others already sitting on the stacks. □

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Anatomy on the move

Cheryl Tickle

Developmental Dynamics. Editor-in-chief Paul F. Goetinck. Wiley. 12/yr. USA \$1,135, Canada and Mexico \$1,255, elsewhere \$1,300.

CLASSICAL embryology and descriptive morphological anatomy spawned modern developmental biology. The American Association of Anatomy has recognized this new offspring and has bravely launched *Developmental Dynamics* to take the place of the *American Journal of Anatomy*. The leading articles and general planning of the new journal give the impression of a change for the better that has been entered into enthusiastically. It would therefore be churlish not to wish this venture well.

The title was presumably chosen to convey the essential movement and change that is so typical of embryos. Also it contrasts with the static images often conjured up by the word 'anatomy'. The journal features papers that describe the types of work that are now commonplace in anatomy departments. One may come across descriptive papers about neural crest migration or find details about the control elements of a homeobox gene. The work may involve zebra fish, *Drosophila* or rats and mice, although plants do not fall within the remit. The journal

has also commissioned reviews and instituted a series of reprints of embryology classics. (The first in the series, V. Hamburger and H. L. Hamilton's article on the stages of chicken development, provided a welcome opportunity to replace worn-out photocopies.)

Is there a need for another journal devoted to developmental biology? It is certainly true that the two main journals devoted exclusively to developmental problems and embryos, *Development* and *Developmental Biology*, seem to be increasingly pressed for space. So another outlet for this rapidly expanding and exciting field could be useful. But it is difficult to see any precise niche that will be uniquely filled by *Developmental Dynamics*. The journal seems to match existing standards of speed and production, and it offers authors a free colour plate, bringing it into direct competition with more established journals. It is certain that in one sense of the phrase, anatomy needs *Developmental Dynamics*, but we will have to wait and see whether developmental biologists need it. □

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Engulfing fields

Brendan J. R. Whittle

Mediators of Inflammation. Editor-in-chief I. L. Bonta. Rapid Communications of Oxford. 6/yr. £240, \$409 (institutional); £90, \$150 (personal).

MEDIATORS OF INFLAMMATION presumably hopes to engulf much of the experimental work on the inflammatory process by soliciting papers from many scientific areas, including immunology, pharmacology, biochemistry and cell biology. It also intends to publish clinical contributions. A reasonable mixture of disciplines is indeed well represented. This is one of the strengths of the journal.

With many even more specialized journals now available, each of which focuses on a distinct discipline or on a particular class of inflammatory mediator such as the prostanoids, leukotrienes or cytokines, the advantage of such a multidisciplinary journal is readily apparent. Moreover, the journal can expect to flourish even when individual mediators fall from fashion (as they inevitably do) and are replaced by new families of biologically active substances (followed rapidly by the appearance of a related specialist journal).

The nature and breadth of recent contributions are healthy for such a young journal. The scientific standard, however, is variable, perhaps reflecting the differences in rigour of the various disciplines as much as a slack editorial policy. Still, this should not excuse the lack of statistical evaluation in some of the papers appearing over the past year. Very few of the latest papers fall far short of a fully worthwhile standard, however. The invited reviews are useful.

One of the selling points of the journal is its goal of rapid publication, aimed at 90 days. Although the speed varied in the earlier issues, the most recent issue contains papers published well within the intended time. The style, layout and typeface are attractive and the quality of reproduction of figures and original photomicrographs is excellent. The personal subscription rate is not too excessive. Affiliation of the journal with an appropriate scientific society might be an effective way of ensuring wider distribution.

The journal should fill a worthwhile place on the shelves of those interested in any aspect of the mediators of inflammation. Competition for really excellent papers will, however, be fierce. □

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Scenes from the corridor

Stuart Sutherland

Current Directions in Psychological Science. Editors S. Scarr and C. R. Gallistel. Cambridge University Press. 6/yr. \$125, £69 (institutional); \$63, £35 (personal).
The International Journal for the Psychology of Religion. Editors L. B. Brown, Ralph W. Hood Jr and H. Newton Malony. Lawrence Erlbaum. 4/yr. USA and Canada \$125, elsewhere \$150 (institutional); USA and Canada \$35, elsewhere \$60 (personal).

JUST as British industry suffers because managers seek short-term profits at the expense of larger long-term gains, so British (and indeed American) psychologists are obsessed by immediate productivity instead of thinking carefully about what projects are likely to be of most value in the long run. Many are so reduced to crossing the i's and dotting the t's of others' work that, on average, research papers are cited only about once after publication. Nowadays, it is admittedly extremely difficult for anyone to take an overall view, for the subject has become so fractionated that most workers know only of what is going on in their tiny corner of the field.

Current Directions in Psychological Science sets out to ameliorate such specialization by presenting short reviews of topics across the whole of psychology and aiming to "keep technical language to a minimum". Unfortunately the first page of the first issue belies the editors' intent, for on it R. J. Sternberg and T. I. Lubart write: "One of the less contextualised approaches is the structurally oriented *psychometric approach*". They proceed to give an account of creativity of such banality that it makes Arthur Koestler's outpourings on the topic seem like the work of Einstein. Their basic contention is that not all original ideas turn out to be of value. By calling this platitude "The Investment Theory", they invite others to take them seriously: the word "theory" when used by psychologists usually signifies that they have nothing to say.

In fact, this article is much the most disgraceful in the issues reviewed. Many of the other contributors give an accurate and clear account of the topics with which they deal and they demonstrate that at least some psychologists are advancing the subject. R. A. Rescorla shows that the learned associations made by animals are much more complex than anything dreamed of by Skinner or even Pavlov; N. Graham gives a good review of the "binding problem" (how the separately detected features of an object are recombined to give a representation of the whole object); and H. Gotlib provides a thoughtful discussion of different theories of the psychological origins of depression.

In the realm of personality, psychol-

ogists are increasingly trying to account for individual differences in evolutionary terms. There is convincing evidence that sexual jealousy is more readily aroused in women by the emotional commitment of their partner to another woman than by mere sexual infidelity, whereas for men it is the partner's sexual act that fuels jealousy. The evolutionary interpretation is obvious.

Despite the catastrophic first article, this journal is for the most part clear, accurate and readable. Any psychologist who wants to know what goes on in the laboratory down the corridor should invest the very small sum required.

The International Journal for the Psychology of Religion represents yet a further specialization in psychology. Its main articles tend to be weak on theory: indeed some of them are mere hand waving. It is easy enough to derive reasons why people believe in God — after all,

someone other than Stephen Hawking must have invented the Universe, the promise of Heaven (though not available in all religions) is attractive and if you happen to be a Freudian you can always posit the need for a father figure with far better qualities than your real father. But none of the authors tackles such outstanding questions as why people need religious ceremonies or rites of passage. For the curious, there are some interesting titbits. For example, an American study of 1,000 Roman Catholic priests found that 40 per cent were practising homosexuals, while a further 20 per cent were active heterosexuals.

Many of the articles are followed by critical comments: these are usually clear and informative and provide an understanding that cannot be gained from the main papers. The book reviews are excellent. Although the individual issues are rather slim, the price is reasonable and the journal should appeal to anyone interested in the serious study of religion or gossip about its adherents: it would have had the imprimatur of William James himself.

Meanwhile, someone somewhere is surely planning an even more esoteric publication, *The Nationwide Journal of the Psychology of Psychologists*. □

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European snoozepaper

Mary A. Carskadon

Journal of Sleep Research. Editor Jim Horne. Blackwell Scientific. 4/yr. USA and Canada \$151, Europe £92, elsewhere \$101.

PUBLISHED on behalf of the European Sleep Research Society and edited by the estimable Jim Horne of Loughborough University of Technology, *Journal of Sleep Research* has quickly established its niche in the scientific literature of this multidisciplinary field. Unlike the short-lived European periodical *Waking and Sleeping* of the 1970s, *JSR* seems destined for an important continuing role. A distinguished panel of ten associate editors — all prominent members of the European Sleep Research Society — is complemented by an equally esteemed 52-member international editorial advisory board, although there could be more female representation on these panels.

JSR maintains a distinctly international outlook and representation. In the first five issues, 55 per cent of articles emanated from European laboratories, 25 per cent from the United States and the rest

from Canada, Israel and Asia. For members of the US sleep research community, *JSR* provides a much needed immediacy of access to and a more intimate link with European colleagues. An important way in which the journal supports an international audience is through its reduced subscription fee to members of any "national sleep society".

Careful editorial screening has resulted in consistently high-quality science. For example, the second issue presented proceedings of a workshop on "concepts and models of sleep regulation". Published within a year of the workshop, the papers in this issue give an up-to-date overview of current theoretical perspectives. One paper unfortunately overlapped to a great extent with an original investigation that appeared in *JSR*'s first issue — this is a hazard of guest editorship. *JSR* has published two "symposia", the first on serotonin and the sleep-wakefulness cycle, and the second on dream research methodology. The five-paper serotonin symposium is excellent, but the limited scope of the dream symposium is disappointing.

Original articles and "fast-track short papers" are generally first-rate and span the field's multidisciplinary spectrum, including studies from comparative biology (cockroach, dolphin, marmot), neurology and the neurosciences, biological rhythms, psychopathology, methodology, endocrinology, regulatory physiology, gerontology, cognitive science and epidemiology. Although sleep-disorders medicine is not entirely eschewed, one feature that distinguishes *JSR* from the other major journal devoted to sleep (the US periodical *Sleep*) is its more limited presentation of disease-oriented papers. Furthermore, such papers are more likely to focus on mechanism than treatment. *JSR* has also published a consensus position paper (on sleep studies for sleep-breathing disorders) from the European sleep community. Such reports provide a valuable frame of reference for the global community of sleep clinicians. In summary, *JSR* is off to a superb start. □

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Aquatic outlets

David R. Livingstone

Molecular Marine Biology and Biotechnology. Editor-in-chief Dennis Powers. Blackwell Scientific. 6/yr. USA \$160, Canada and Mexico \$170, elsewhere \$190 (institutional); USA \$70, Canada and Mexico \$80, elsewhere \$100 (personal).

Fish and Shellfish Immunology. Editors A. E. Ellis and M. F. Tatner. Academic. 7/yr. £135, \$255.

AQUATIC biologists will welcome the establishment of these two periodicals that gather together material previously scattered among the literature or tagged with the term 'comparative' and given a hard time by mainstream mammalian-orientated journals. The editorial effort and standard of work evident in both will undoubtedly give a boost and some extra rigour to the fields covered.

Both journals are attractive and distinctive in appearance, and publish primary research papers, occasional reviews and short communications. Most of the work reported in the first volume of both *MMBB* and *FSI* is on fish (75 and 90 per cent respectively, including a special *MMBB* double issue on transgenic fish), which may reflect research funding as much as anything else.

The launch of *MMBB* is particularly timely. Molecular biological methods are

being applied to more and more areas of science, yet until now there has been no obvious single publication outlet for aquatic scientists using such techniques. *MMBB* aims to publish papers that apply these methods to biological and ecological questions. The editorial board is predominantly from the United States, but recruitment from other parts of the world is promised. The large format allows the inclusion of many photographs (such as western blots) and easily read and extensive gene sequences. Reproduction of detailed micrographs and colour photographs is excellent. Although most of the papers in the first issue are on DNA and RNA, a fair number also deal with protein or cell-biological studies, including enzyme purification and characterization. Papers of two to ten pages are requested. Although 'biotechnology' is in the journal's title and a stated aim is "to enhance the utilization of aquatic resources", it is to be hoped that applied matters do not come to overshadow the basic science.

FSI aims to present studies on the basic mechanisms of both the specific and non-specific defence systems of fish and shellfish and the application of these findings to the aquaculture industry. It has a multinational editorial board, and to a limited extent overlaps with *Disease of Aquatic Organisms*, although the new journal's focus is exclusively on immunology. *FSI* will particularly help to galvanize and stimulate work on invertebrates. □

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In the incubator

John Blaxter

Fisheries Oceanography. Editor-in-chief T. R. Parsons. Blackwell Scientific. 4/yr. USA and Canada \$157, Europe £95, elsewhere £105 (institutional); USA and Canada \$89, Europe £55, elsewhere £60 (personal).

THE birth of a journal follows a now familiar course: a distinguished scientist is appointed as editor (midwife) and is supported by a heavyweight editorial board of assistant midwives; the baby no doubt undergoes a gestation period of at least nine months but is underweight at birth and subsequently shows signs of uneven growth and imperfect development; all being well (that is, if libraries have adequate funding), a viable juvenile results. *Fisheries Oceanography* is no exception, and is presumably now in the incubator stage.

Publishers take well calculated risks in

starting a new journal, knowing that perhaps 600–700 institutional subscribers are needed for it to make a profit. The stated aim of *Fisheries Oceanography* is to publish original papers, reviews and short communications on research relating the production and dynamics of fish populations to the marine environment. The editor-in-chief is aided by two associate editors and an impressive editorial board of 21 scientists. In the four issues available for review, many of the papers can indeed be said to contain fisheries oceanography data but several cover purely fish biology and one purely oceanography. There is a fairly strong bias towards the North Pacific, reflecting, perhaps, the geographical location of two of the editors. Only one paper is on shellfish.

The standard of production is fair. The double-column format is not ideal for figures, many of which are too small and sometimes badly reproduced. Although the publishers have been parsimonious in this respect, they have allowed the publication of several colour plates, but it is not clear whether the authors have been charged for these. Colour can certainly be useful with the current vogue in satellite imagery and complex synoptic charts. At present, papers are accepted within two to three months of receipt and published within a few months. The editors are to be congratulated on getting their referees to report back within such a short time and the authors to revise their papers equally speedily.

Does this journal fulfil a need? A natural home for even a diffuse topic such as fisheries oceanography is useful, but there are many well established journals that are equally appropriate (for example, *Canadian Journal of Fisheries and Aquatic Sciences*, *Fishery Bulletin US*, *ICES Journal of Marine Science* and *Marine Ecology, Progress Series*). Fisheries oceanography is a long-standing topic. Why do not publishers confine themselves to commissioning journals in new fields?

Subscription prices are set at about the right rate — they will not deter librarians yet allow for some increase should the journal expand. In the surprising absence of an introductory editorial we are not party to the ambitions of the publishers and editors. Nor are we given the rationale for excluding lake fisheries and limnology, coverage of which would, in fact, have given the journal a wider and better remit. The danger is that librarians will wait to see how the baby progresses before taking a stake in its development. This means several years of subsidy by the publishers and some faith on the part of putative authors. □

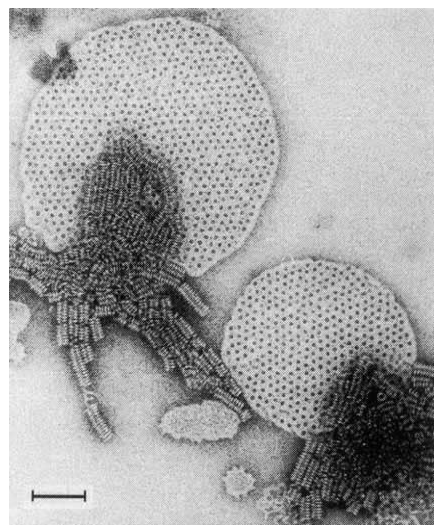
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Surrogate cells

A. D. Bangham

Journal of Liposome Research. Editor Leaf Huang. Dekker. 3/yr. \$225 (institutional); \$112.50 (personal).

SMECTIC mesophases (layer lattices of amphiphilic molecules in water, alias 'myelins' and now called 'liposomes') would have been found on our planet long before covalently linked nucleic or amino acids; in other words, membranes came first. Some 30 years ago, I and my colleagues recognized the fact that such structures, made with phospholipids of biological origin, spontaneously formed systems of closed membranes when placed



Cholesterol-containing multilamellar liposomes challenged with a dilute solution of saponin and negatively stained with sodium phosphotungstate. From A. D. Bangham, *Advances in Lipid Research* 1, 65–104 (1963). Scale bar, 1,000 Å.

in water — that is, surrogate cells.

Early papers describing their structure and properties were despatched, of necessity, to molecular, biochemical and biophysical journals for peer review and acceptance depending on their particular slant. Biological membranes were in vogue in the 1960s and the attributes of their bimolecular simplicity marvelled at; the (passive) properties of all biological cell membranes were revealed! But the main impetus for additional research arose from the idea that liposomes might be used as nontoxic, biodegradable, addressable envelopes to deliver potent drugs precisely and quickly or, alternatively, surreptitiously and slowly.

My liposome database (LIFO Avestin Inc., Ottawa) now contains about 14,000 references going back to 1965, with additions running at roughly 1,000 a year. This new journal should have no difficulty in selecting "high quality articles

in the area of liposome research", the avowed aim of the editor and his board.

Volume 2 is now in the, perhaps, neutral hands of an editor based within a university department. There is also a generous representation on the editorial board of scientists working in both commercial and academic fields.

I approve of the "Forum" section, in which a panel of interested workers in a particular field is invited to comment on a group of published papers. The comments amplify the salient points made in the seminal articles under scrutiny and, in the long run, will help to defuse controversial issues.

With the choice of camera-ready text, publication time should and would seem to be acceptable, although one wonders, in view of the ease with which one can alter text with wordprocessors, why there is such a great variation in font style. It does, however, add flavour. □

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Cellular therapies

Kathryn Wood

Cell Transplantation. Editor-in-chief Paul R. Sanberg. Pergamon. 6/yr. £210, \$325 (institutional); £57, \$88 (personal).

TRANSPLANTATION is a growing discipline. If the launch rate for new journals covering different aspects of the field is a reflection of the rate of progress in the development of strategies that will ultimately find application in clinical practice, then exciting times are ahead.

The transplantation of cells rather than whole organs is by no means a new idea. But whereas the transplantation of solid organs is now accepted as a useful therapeutic strategy, transplantation of bone marrow is the only clinical discipline in which single cells are transplanted on a regular basis. The current lack of routine clinical application of cell transplantation does not reflect a lack of interest in the potential of this approach; hence the advent of *Cell Transplantation*, which aims to provide a forum for discussion of transplant research at the cellular level.

There are many disease states in which cell transplantation might eventually be used as a form of treatment, including diabetes, certain forms of hepatic insufficiency, some neurological disorders and muscular dystrophy, to name but four. And an even more exciting future therapeutic prospect is the transplantation of genetically modified cells. In the first few issues, the journal has published original experimental and clinical

studies in each of these areas and others, including reports of advances in the technology for growing isolated cells *in vitro* and their encapsulation in artificial membranes. The quality of the papers is improving. But in attempting to cover not only all the potential clinical applications but also the developments in the many technologies that will be required for the successful clinical application of cell transplantation, the scope of the journal may be too broad to provide comprehensive coverage.

The editorial board consists of distinguished workers and is subdivided into areas of expertise. An editor with specialist knowledge has been designated for each section, a policy that ought to ensure the quality of the papers. So far, the content has varied from issues containing only original papers to issues exclusively consisting of invited reviews. Because of the potential clinical application of the research, the editor has chosen to include a section of abstracts from recently issued US patents. In some issues there is also an editorial section for the discussion of controversial issues, such as the use in the United States of fetal tissue for clinical transplantation research. The editors should now cater for wider international interests by broadening the focus to other areas of the world.

The advent of this journal was well timed. With good editorial guidance, *Cell Transplantation* should develop to become the leading publication in this specialized area. □

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Sensible medicine?

Ian Gibson

Antisense Research and Development. Editors James Hawkins, Arthur M. Krieg and Cy. A. Stein. Liebert. 4/yr. USA and Canada, \$121; elsewhere £161.

IF there are still doubters who believe 'antisense' is too clever by half or that it just does not work, then they should take a look at a review in a recent issue of this journal of a US meeting on the subject:

The conference ended on a very enthusiastic note about the future of nucleic acid-based therapeutics in medicine. Based on the data presented at the conference, it became clear that many of the major objections that some scientists had raised in the past about antisense and the other technologies are rapidly becoming moot. Oligonucleotides can be quite stable

in the body; a number of exciting *in vivo* experiments have shown remarkable therapeutic benefits; delivery of oligonucleotides to cells in an active form occurs at reasonable concentrations; cost of manufacture is falling rapidly; and most oligonucleotides seem relatively non-toxic with large therapeutic windows".

Antisense Research and Development was launched in 1991 and it is now an outstanding success. Results from the early days, on the stability and uptake of particular compounds, and on their resistance to nucleases in the serum, are now being re-examined in the light of antisense technology. Serious scientific and therapeutic questions are being asked about delivery and uptake of oligodeoxynucleotides, their metabolism, mode and specificity of action, and localization in cells. The design of new modified stable compounds is taxing chemists, drug companies and young businesses. Many articles covering all these research fields have been scattered among a whole range of journals, so a publication that pulls the work together under one cover is to be welcomed.

A useful feature of the journal is the comprehensive reporting of conferences and the bibliography of the burgeoning antisense literature. There are also guest editorials and brief communications. Provided that drug companies remain interested in antisense therapeutics, I foresee a happy future for the journal. □

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Genetic markers

David Skibinski

Molecular Ecology. Editors Terry Burke, Ray Seidler and Harry Smith. *Blackwell Scientific*. 6/yr. USA and Canada \$335, Europe £205, elsewhere £225 (institutional); USA and Canada \$61, Europe £35, elsewhere £41 (personal).

WHAT would a molecular-biologist time-traveller from the early 1960s ask when confronted with a journal called *Molecular Ecology* that carries photographs of dragonflies or colourful plants or mysterious diagrams (dot blots) on its cover. "Have the frontiers of science been pushed so far back and reductionism been so successful that ecology is now explained by the interactions of molecules?" Or perhaps: "Are ecological theory and principles now being applied to the molecular physiology of living organisms?" A geneticist time-traveller from a decade later might more easily guess the truth — that the DNA revolution has brought a plethora of techniques for measuring

genetic variation. This is the secret of this journal.

Some of these techniques are marvelous, and have opened up areas of population biology, such as paternity analysis in birds or the fate of genetically manipulated microorganisms released into the environment, that were difficult or impossible to investigate with protein electrophoresis. A generation of population biologists with enthusiasm for ecological problems dulled by the craving for the next good gel or autorad will need ever-increasing journal space. *Molecular Ecology* provides a home for this work and is therefore likely to have a good chance of survival.

The editors admit that the term molecular ecology is both vague and trendy. Rather than imposing their own definition, they instead hope that the scope of the journal will be determined by the papers submitted. Nevertheless, there are guidelines, with emphasis on the molecular investigation of natural and introduced populations and their environments and on the ecological implications of the release of recombinant organisms. Over the first 18 months, DNA fingerprinting, restriction fragment length polymorphism analysis, polymerase chain reaction and random amplified polymorphic DNA are the techniques very much to the fore (though allozyme analyses are not banned). The species are scattered across the entire range of living organisms with genetically modified organisms and microorganisms holding their own. On the theoretical side, disciplines related to population genetics dominate. The time-traveller might be disappointed to see little on the ecological implications of variation in patterns of gene expression.

Technique is exalted, yet it is used in almost all contributions only as a means of discovering genetic markers. Perhaps no more can be expected at the moment, but it will be important for the editors to guard against the journal becoming a dumping ground for papers using trendy techniques but with little theoretical interest. However, the juxtaposition of papers on plants, animals and microorganisms investigated using the same molecular techniques is interesting and instructive.

The journal is well produced and has an attractive format. Contributions in the form of full papers, short communications and technical notes are permitted. There are also extremely useful mini-reviews of specific techniques. What comes across clearly is the ambition and enthusiasm of the editors for establishing a good-quality, rapid-publication journal that is of interest to all population biologists. □

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Blood relations

A. D. Whetton

Stem Cells. Editor-in-chief M. J. Murphy Jr. *AlphaMed*. 6/yr. USA and Canada \$150, elsewhere \$162 (institutional); USA and Canada \$85, elsewhere \$97 (personal).

Journal of Hematotherapy. Editors-in-chief Adrian P. Gee and Nancy H. Collins. *Liebert*. 4/yr. USA and Canada \$114, elsewhere \$154.

DURING the past few years there has been great progress in our understanding of the mechanisms underlying the regulation of haemopoietic cell proliferation and development. The results of this research have rapidly found clinical application in haematology and oncology, for example in developing new approaches to cancer treatment, bone marrow transplantation and immunotherapy.

Although both journals are very much concerned with haemopoietic stem cells, they concentrate on different aspects of haematology. Previously published as the *International Journal of Cell Cloning*, *Stem Cells* is a mixed bag of original papers, book and software reviews and concise reviews of recent advances in oncology and haematology. As with its predecessor, these informative and useful reviews are one of the main attractions of the journal. They are generally of high quality, as indeed are many of the original papers. The scope of the papers, however, is as broad as many haematological and leukaemia research journals; as such there is little to differentiate *Stem Cells* from better established journals.

Journal of Hematotherapy, on the other hand, has gone for a specialized niche. The journal concentrates on the collection, purification, storage and *in vitro* manipulation of haemopoietic stem cells for use in transplantation. This is plainly a multidisciplinary area deeply affected by the recent rapid advances in experimental haematology. The journal achieves its stated goal in that it encompasses practical and theoretical aspects from several disciplines applicable to the *in vitro* manipulation of stem cells. It contains meeting reports, articles on guidelines and regulations, news from companies, readable reviews and (in the main) accomplished research articles. It should be a great aid to those involved in stem cell manipulation for clinical use, while also providing useful background information for nonspecialists. □

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Mankind in the making

Ian Tattersall

Evolutionary Anthropology: Issues, News, and Reviews. Editor J. G. Fleagle. Wiley. 6/yr. USA \$145, Canada and Mexico \$205, elsewhere \$227.50 (institutional); USA, Canada and Mexico \$36, elsewhere \$54 (personal).

PALAEOANTHROPOLOGY is one of those messy sciences that uncomfortably blends many areas of specialization. At its heart a systematic science, it also draws extensively on geochronology, molecular biology, archaeology, functional anatomy, ecology, evolutionary biology, primatology, taphonomy, stratigraphy and a host of other fields. Rarely will one tackle any general problem in human evolution without sooner or later having to delve beyond one's central expertise, an exercise designed to make one frustratingly aware of how difficult it is to keep up with developments in several rapidly moving — and diverging — sciences.

Evolutionary Anthropology aims to help with this problem by providing regu-

but all palaeoanthropologists will benefit from having an easily accessible source of discussion of at least several of these topics, and of others in later numbers.

The journal also contains reports on symposia, book reviews and a correspondence section intended as a forum for debate. The meat of the journal, however, lies in the review articles. These are long

enough to contain a lot of valuable detail, yet they are written at a level that will make them useful not just to professionals but also to graduate students and perhaps to some undergraduates. The articles are copiously referenced and illustrated, and Fleagle has rounded up an impressive roster of authors for the first few issues. If this publication continues as it has begun, and succeeds as it should, future historians will find it an extremely handy record of the evolution of a subject whose varied dimensions are increasingly hard to keep track of. □

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Archaeological meat and veg

Paul Bahn

International Journal of Osteoarchaeology. Editors Ann Stirling and Tony Waldron. Wiley. 4/yr. \$195 (institutional); \$145 (personal).

Vegetation History and Archaeobotany. Editors Karl-Ernst Behre and G. L. Jacobson Jr. Springer. 4/yr. DM248, \$155.

THE field of archaeology is so wide-ranging and all-encompassing — any and every aspect of the human past being fair game — that its every specialized facet can, and no doubt eventually will, spawn its own journal. One can confidently predict the appearance before long of the *Journal of Ethnoarchaeological Taphonomic Studies* and *Shellmidden Quarterly*. But most journals don't come cheap, and impoverished archaeologists and financially stretched libraries need powerful incentives to take out new subscriptions.

These two new journals have a certain amount in common: high quality production with good photographs and line drawings (with occasional colour diagrams in *Vegetation History and Archaeobotany*), a readable typeface, double-column text and a high price for a total of 367 pages and only 260 pages respectively. Both contain research papers, review articles and short reports. Both have their contributions peer-reviewed and seem to have a remarkably rapid process of acceptance: only a week or two in some cases (though much longer in others), followed by almost immediate publication in the case of *International Journal of Osteoarchaeology*. One wonders whether this reflects extreme efficiency, or rather desperation and a dearth of incoming manuscripts.

IJO, which began in 1991, also contains one or two book reviews and an occasional leading article and obituary. Its striking

logo, a human skull and a carnivore skull, is no doubt intended to underline the journal's aspiration to be a forum for the publication of papers dealing with all aspects of the study of human and animal bones from archaeological contexts. But the human skull is above the animal, and similarly the journal's contents so far have been very heavily dominated by human palaeopathology.

Indeed, were it not for very occasional contributions on animal remains, a bit of taphonomy and some aspects of ageing and sexing, a scan of these bones would lead one to see the journal as devoted entirely to past human ailments. In theory, this should not make it any less interesting to the average archaeologist, but in practice it does: it is an odd fact that, in most courses on archaeology, a subject that purports to study all aspects of the lives of our forebears, the human skeleton is greatly neglected. As a student I was taught animal bones in some detail, but never given a bit of dead human to look at. A textbook of archaeology that I wrote with Colin Renfrew a few years ago (*Archaeology: Theories, Methods and Practice*, Thames and Hudson, 1991), was greeted with surprise and even alarm in some quarters, especially the United States, because it devoted a whole chapter to human remains. Although humans were the principal actors in the play we seek to revive, it has traditionally been left to nonarchaeologists to study the human remains we dig up.

Seen from that perspective, *IJO* is a welcome arrival if it makes archaeologists more interested in studying human remains, but the journal has so far failed in its aim of covering animal-bone studies in equal depth. The editors are fully aware that these are underrepresented, and plead in a recent issue for more to be



Hominid skulls from East Turkana, Kenya. From left, *Homo habilis*, *Homo erectus* and *Australopithecus robustus*.

lar critical reviews of developments in all the subdisciplines that bear on our understanding of our own evolution. Only a year (six slim issues) down the line, it's still a bit early to say whether the journal will have any great influence on the fragmentation of palaeoanthropology that subspecialization inevitably entails and that the editor, John Fleagle, deplors; but it's already clear that the journal fills a niche that has stayed vacant too long. Its first three issues alone contain reviews on subjects as diverse as human behavioural ecology, the assessment of body size in early hominids, the archaeology of modern human origins, uranium-series dating, variable tandem repeats, the vexed question of *Homo erectus* (Asian and African? just Asian?), new views on primate origins, prehistoric cannibalism, and deception among primates. Some of these reviews are inevitably blander than others,

submitted. But it will be an uphill struggle, as the new journal is not yet well known (I had never heard of it before being asked to write this review), and animal bone specialists have had their own outlet, *Archaeozoologia*, for some years now, as well as the *Journal of Archaeological Science* and other more general titles.

Vegetation History and Archaeobotany, which began in 1992, also aims to encompass everything in its field on a worldwide scale, but with markedly greater success so far. Its declared agenda is to cover past forms of vegetation, the elucidation of palaeoclimatology (Quaternary and especially Holocene), the palaeoenvironment of archaeological sites and the evolution of agriculture. It wishes to combine the traditional concerns of vegetation history (the development, migration and expansion of species) with a special emphasis on past vegetation patterns and the changes caused by human interference, a reconstruction of how the cultural landscape came into being. The tools for such studies are essentially palynology and the analysis of botanical micro- and macro-remains, and the journal intends to keep up to date with new techniques and technical developments.

In short, the editors' aim is to gather together the material that used to be in archaeological journals of limited regional circulation, and produce a research resource of general interest. One valuable inclusion is an annual bibliography on cultivated plant remains from archaeological sources — continued from the 22 that appeared in *Kulturpflanze* up to 1990 — with species arranged alphabetically by Latin name for quick reference. This feature already makes the journal invaluable to specialists in vegetation history, but there are also detailed palynological reports (with fold-out diagrams), studies of plant remains from archaeological sites or vessels and, as the editors hoped, plenty of papers on human disturbance of vegetation in different places and periods.

Both journals are clearly designed to fill gaps and find a market by being interdisciplinary, but only *VHA* has really achieved this so far. The 'archaeo-' in both titles indicates that they are fundamentally targeted at the wide world of archaeology — indeed *VHA*'s logo is a drawing of ears of Neolithic wheat from Switzerland. But although the average archaeologist will certainly find items of interest in individual issues, there are probably not enough to warrant a subscription. For the present, apart from already committed archaeological specialists, *IJO* will appeal primarily to anatomists, medical students and physical anthropologists, while *VHA* will attract environmentalists, botanists and climatologists. □

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Yams and poisons

Simon P. Wolff

Natural Toxins. Editors-in-chief John W. ApSimon and J. David Miller. Wiley. 6/yr. USA \$195, Canada and Mexico \$255, elsewhere \$277.50 (institutional); USA, Canada and Mexico \$97.50, elsewhere \$127.50 (personal).

I LIKE to ask my students on drug metabolism and toxicology courses why yams synthesize impressive quantities of contraceptive steroids and why tetrahydrocannabinol (THC) is a constituent of hemp. Then we talk about the evolution of drug-metabolizing systems in the context of the vast range of chemical defences (many very subtle) that plants possess to stop predation. Regrettably, studies of natural toxins, from plants or animals, seem not to be brought together in a forum allowing interdisciplinary study.

So I was pleased to see the production of a new journal that aims to bring the study of these fascinating compounds under one roof. *Natural Toxins*, it seems, is concerned with the occurrence, isolation, identification and characterization of natural products with toxic activities, focusing on human toxicology but generally covering immunology, pathophysiology, biochemistry, chemistry, nutritional significance and potential therapeutic applications. The journal is of high quality and reasonably varied in the subject matter it attracts. It is nice to know what's new in scorpion intoxication, ink-cap poisoning (unpleasantly like Antabuse when eaten with alcohol) and 'Red Tide' poisons.

One complaint from the perspective of a generalist is that the journal seems very biochemically descriptive in orientation, with too little ecology — the most interesting part of interspecific biological warfare. I also worry about the tone set in the first leading article, in which Bruce Ames repeats his now familiar tales that man-made chemicals are unlikely to represent a health threat compared with, say, natural pesticides and that there is a real problem with chronic toxicity testing. The discussion strikes me as an oblique way of introducing a journal of such wide ambition and potential interest and as not really pertinent. For I doubt that the journal is primarily aimed at the human toxicologist, given the range of veterinary subject matter (horse and pig poisoning) in the first issues. The error is compounded in the second issue with a leading article on a similar theme (human risk assessment of natural poisons in food), so there has to be a worry that the broad 'Aims and Scope' is a vague wish rather than an explicit editorial policy.

Perhaps later issues will come with a

wider range of invited reviews in order to elicit a suitable range of original papers. The journal has real potential, however, and I look forward to reading detailed answers to the questions I ask my students. I tell them that yams make contraceptives to control population levels of local grazing mammals. I also tell them that hemp contains THC because cattle, unlike humans, don't like being stoned. But I worry that these explanations are fanciful and hope that *Natural Toxins* will one day publish papers giving me an accurate answer. □

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Gone with the wind

John Postgate

Microbial Releases. Editor-in-chief Walter Klingmüller. Springer. 4/yr. DM390, \$244.

ALTHOUGH dates of receipt of manuscripts are not printed, this journal publishes promptly papers bearing on the release of microbes, both manipulated and non-manipulated, into the environment. Original papers tend to be short — five to eight pages — and there are a couple of 'Short Communications' (three to four pages). Each issue begins with a 'Review' or a topical 'Status Report'; there are also 'Commentaries' (brief accounts of recent scientific meetings) but no editorial comment, letters, book reviews or correspondence. The Harvard reference system is used; layout and quality of type and illustrations are up to Springer's usual high standard.

Original papers cover topics such as the survival of natural and recombinant bacteria on leaves, in water and in soil; gene transfer in native and amended soil; the fates of spores or recombinant bacteria in animal intestines (from which they could inadvertently be spread); *lux*⁺ recombinant bacteria as environmental probes or monitors; model work on the dispersal of phyllosphere bacteria by raindrops; overproduction of indolylacetic acid by a recombinant *Azospirillum*; exploitation of a recombinant β -toxin-producing bacterial endophyte to protect corn plants from the European corn borer; and direct extraction of DNA from soil. One review deals with artificial encapsulation of bacteria, another with survival and detection of bacteria in the environment; the one status report concerns the remarkably

successful use of recombinant vaccinia virus to control rabies in Belgium. Commentaries summarize meetings on "biosafety" monitoring, and nitrogen fixation in Russia.

Judging quality is a very subjective matter. As one-time chief editor of a mainstream microbiology journal, I would have rejected a few of these papers as too preliminary or inconsequential. I would also have been more meticulous over terminology, avoiding, for example, the use of the term 'viable' in two different senses. Rapid publication need not mean sloppy writing. But, as if to balance some indifferent editing, several papers are both fascinating and impeccably presented.

Microbial releases are a matter of some public anxiety, and of considerable research activity, and it helps all concerned to have relevant papers gathered together rather than scattered among diverse journals of general and applied microbiology. But releases will become routine and anxieties will recede; whether the niche occupied by this journal will remain for long is another question. □

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■ As *Nature* went to press, it learned from Springer that *Microbial Releases* is to cease publication at the end of the year.

Slices of the synthetic cake

Leslie Crombie

Natural Product Letters. Editors-in-chief Atta-ur-Kahman and E. Wenkert. *Harwood Academic*. 4/yr. \$258 (institutional); \$65, £43 (personal).

SINCE its inception as a recognized branch of science more than a century-and-a-half ago, the study of natural products has provided an important dynamic to organic chemistry. This journal claims to cover all aspects of research in the chemistry and biochemistry of naturally occurring compounds of land and sea, and of plants, microbes and animals, along with structure elucidation, synthesis, experimental biosynthesis and methods used in these areas. Research papers on the boundary between chemistry and biology — fermentation chemistry, plant tissue investigations and so on — are also considered. This is indeed a large slice of the organic chemistry cake.

The wide coverage places the journal in direct competition with many established outlets for the type of material it wishes to

attract, and prospective authors and readers need to assess the advantages or disadvantages of the new publication relative to other journals. I was hampered by a shortage of information in making comparisons. For example, it is not clear to me if submissions are independently refereed, a process I regard as essential in any good journal.

The first four-part volume is of modest length (310 pages) and is spread over 1992 and 1993. Except for the year of publication, however, each issue is undated and it is impossible to work out average publication times. The first volume contains 52 communications, of which about 23 are primarily synthetic in character and might well fit into specialist synthesis journals.

Apart from the external covers, the camera-ready pages resemble the long established *Tetrahedron Letters*. But authors in that journal are restricted to four-page preliminary accounts of urgent material. Some of the communications in

Natural Product Letters (which allows authors a maximum of eight pages) would come in this category, and are certainly of good quality, but others are of lesser urgency and do not, I think, deserve preliminary publication. Some are full papers, complete with all experimental details. Then again, some lie part way in between. Blurring the distinction between preliminary communications (which *Letters* usually implies) and complete, or partly complete, papers seems to me to be undesirable; some standardization would be helpful.

Some clear editorial thinking about the focus of *Natural Product Letters* and its place in the literature now seems due. Library budgets are everywhere under strain, and this journal will have to make a strong competitive case if it is to be bought by chemistry departments. □

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Structure and technique

Robert Tycko

Solid State Nuclear Magnetic Resonance: An International Journal. Editor-in-chief J. Klinowski. *Elsevier*. 6/yr. DFL446, USA and Canada \$241.

THE proclamation that nuclear magnetic resonance (NMR) is dead has become something of a joke among practitioners of NMR spectroscopy. New technologies and new ideas have produced an explosion of activity in solid-state NMR in the past ten years. The importance of NMR in solid-state inorganic chemistry, catalysis, materials science and biochemistry is now recognized by academic chemistry departments. Solid-state physicists are recalling that NMR can be an essential experimental tool, their memories refreshed by recent NMR studies of high- T_c superconductors, fullerenes and alkali fullerides, organic conductors, charge-density wave materials and other systems that demonstrate the power of NMR as a local probe of the electronic, structural and dynamic properties of solids.

These various forces have brought forth this new specialized journal, "a forum in which results and opinions are exchanged between researchers using NMR methods to study the properties of solid materials and researchers developing NMR instrumentation, experimental methodology, and new theoretical and computational techniques". The journal plans to publish invited reviews, book reviews and announcements in addition to full-length research papers and short communications. The production quality is excellent.

There is no question that solid-state NMR spectroscopists need a journal of their own. Developments in solid-state NMR techniques and technology are often better published in a specialized journal than in a more general publication, where they may miss their proper audience (or run into hostile referees). Even spectroscopic studies of systems that are of wide scientific interest often lead to two types of papers simultaneously, one for people interested only in the systems themselves and one for people also interested in the details of the spectroscopy. The new journal does, however, face some competition, primarily from the *Journal of Magnetic Resonance*, now in its twenty-fifth year. That journal has always had a much broader mission, with most of its papers dealing with liquid-state NMR. But in 1993, it split into two parts, "series A" devoted to information of particular relevance to chemistry and physics, "series B" to biology and biochemistry. It seems possible that series A will tend to attract papers on the solid state. Of course, only time will tell how researchers choose to apportion their manuscripts among the various journals, specialized and otherwise. So far, papers published in *Solid State Nuclear Magnetic Resonance* have been of uniformly excellent quality and of importance to practising solid-state NMR spectroscopists. All indications are that this trend will continue. □

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Manufacturing success

Neil Alford

Materials World. Editor Nuna Staniaszek. *Institute of Materials.* 12/yr. Europe £136, elsewhere \$283.

SEVERAL years ago it was agreed that the British institutes representing the three main branches of materials — metals, polymers and ceramics — should join forces to form an Institute of Materials. This good idea was so straightforward that several obstacles were inevitably laid to delay the merger. Fortunately, common sense prevailed and the new institute was formed in 1992. With it came *Materials World*, the institute's house journal. Today the institute has 19,000 members, all of whom already receive the periodical.

Materials World, which subsumed *Metals and Materials*, *Plastics and Rubber International* and *British Ceramic Journal*, aims to cover all aspects of materials science, and the feature articles are therefore written so as to have broad appeal. So far, I have read each of these with interest: there is indeed a wide variation in topics covered and the standard of the articles is generally high.

In the first issue, for instance, there is an excellent article by R. J. Brook and R. A. D. Mackenzie on nanocomposites, as well as others on ceramic-matrix composites, metal-matrix composites and polymer composites (thus effectively offering something for everyone, whatever their particular discipline). I was especially intrigued by the article by Barry Minton of Schroder Ventures on how to make use of venture capital. In today's difficult economic climate, sensible advice on such matters is probably exactly what the UK materials industry needs. In this regard, John Hodgkinson's informative piece on 'smart' materials is also relevant: there is a surprising lack of work in the United Kingdom on this potentially important development, particularly compared with that done in the United States and Japan.

Indeed, a report on an overseas science-and-technology expert mission to the United States notes the lack of strategic planning in the United Kingdom, a deficiency that the authors lay squarely at the feet of the Department of Trade and Industry. There are similar parallels here to a recent report on the US superconductivity industry. A ray of hope is found in the announcement of the formation of a new committee on smart materials. Such quasi-political comments are plainly in the interests of all materials scientists, and a refreshing aspect of the journal.

Other sections include News, Innovation, Reviews, Education (information on



Polarized light micrograph of the high- T_c superconducting ceramic yttrium-barium-copper-oxide $\text{YBa}_2\text{Cu}_3\text{O}_{6.9}$ (magnification $\times \sim 90$).

courses and so on), Product Focus, Looking Ahead (events in the materials calendar) and Classified (jobs and services). The format is a little like that of *Physics World* but *Materials World* doesn't seem to have the same sparkle (this may simply

be due to the slightly drab and insubstantial paper on which it is printed).

The correspondence section contains some fairly vigorous letters and a fair number of even more robust replies. Such infusion of excitement is essential if students are to recognize materials science for what it is — a wide-ranging and fascinating area of research and development that is crucially important to the success of manufacturing industry. With its admirable aim of being cross-disciplinary and its early success in attracting some excellent features, *Materials World* will un-

doubtedly be of great help in conveying this message. □

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Forging ahead with an ion cast

Stephen Mann

BioMetals. Editor-in-chief G. Winkelmann. *Rapid Communications of Oxford.* 4/yr. £175, \$295 (institutional); £65, \$110 (personal).

BY their very nature, new journals that cater for a multidisciplinary readership often suffer the occasional anxiety attack over a sense of diminished identity. *BioMetals* is a new journal that has been quickly forged from the embers of the first (and last) four volumes of *Biology of Metals* and it is to be hoped that the new name and style give it the distinctiveness it so readily deserves. Unfortunately, the title does not help much, because the number of actual biometals known is very small (Au(0) deposition in bacteria is one example). But the front cover of the journal attempts to offset this ambiguity by informing us that what is really at issue is the role of metal ions in biology, biochemistry and medicine. Originally led by the bioinorganic chemists, new areas of this research are now opening up in disciplines such as molecular biology, toxicology, metabolism and nutrition. It is in this second wave of metal-ion biochemistry that *BioMetals* is leading the way, providing a forum for areas on the periphery of the more traditional studies of the chemical structure, bonding and reactivity of metal-ligand interactions in biological molecules.

The first five issues of *BioMetals*

are encouraging. There is usually a minireview (about ten pages in length) followed by eight or so medium-length (four to ten pages) research papers. The review papers are excellent and, in my opinion, crucial to the future success of the journal. The research papers are generally of high scientific quality and the data (figures and tables) are well laid out. A wide range of subjects is presented, including biocoordination chemistry, metal-ion transport, toxicity and resistance, physiology and bioremediation. Studies on the role of metal ions in toxicology seem to be the most common. The papers have an international scope with India, the United States and Germany well represented. In some issues there is a page at the back on future conferences but nothing so far on new books or recent highlights in the field.

BioMetals is published by Rapid Communications of Oxford, which, as the name implies, guarantees that with fair sailing one can submit in March and be home and dry by the summer. This full-steam-ahead approach is considered to be an important aspect of the journal; and the editors point out that refereeing standards will not be compromised. It is a little early to be able to assess whether this will be the case, but the first two volumes bode well for the future. □

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New materials in old niches

Martin R. Bryce

Advanced Materials for Optics and Electronics. Editor-in-chief D. J. Cole-Hamilton. Wiley. 6/yr. \$595 (institutional); \$445 (personal).

Molecular Engineering. Editors J.-M. Lehn, J. Maruani and J. Zyss. Kluwer. 4/yr. DFL382.

Molecular Materials. Editor-in-chief L. M. Blinov. Gordon and Breach. 4/yr. \$384 (institutional); \$85, £57 (personal).

CONTEMPORARY materials science crosses the traditional boundaries of biology, chemistry, physics and engineering. Each of these three new journals is aimed at a multidisciplinary readership, with something for anyone interested in the synthesis, characterization and device applications of molecular or polymeric materials. The unifying theme of the journals is that the materials discussed should possess specific properties (biological, chemical or physical) related to the burgeoning discipline of information technology. In the issues that I surveyed, papers cover a wide range of topics such as liquid crystals, evaporated thin films, Langmuir-Blodgett films, charge-transfer interactions, nonlinear optics, ferroelectrics, laser dyes and processing of device structures. The new journals will have to compete not only with one another but also with established interdisciplinary materials publications such as *Advanced Materials*, *Chemistry of Materials*, *Journal of Materials Chemistry* and *Macromolecules*, all of which surpass the new publications both in the quality of science reported and in the layout of the articles. From the viewpoint of a chemist, I fail to see the justification for even one new materials journal, let alone three. Many of the articles in my own area of knowledge were of mediocre quality, reporting results that were largely routine. There were, however, a few refreshing exceptions.

Advanced Materials for Optics and Electronics has been created by combining *Chemtronics* and *Molecular Electronics*. The subject matter is divided roughly equally between organic and inorganic materials, with an emphasis on physical characterization, spectroscopy and engineering studies. Each issue contains, on average, six original research papers, which makes it a very expensive publication. There is scope to publish short communications, but there were no such pieces in the issues I looked at. The average time between acceptance of the manuscript and publication is three to four months. Colour figures are used to good effect in some articles.

Molecular Engineering is published only four times a year, which will inevitably lead to some delays in publication. The review articles and research papers

are concerned with both experimental and theoretical studies, with an emphasis on organic and bioorganic systems, including membranes. Several papers concern semi-empirical studies. The presentation is variable: more than one paper contained several typographical errors and, incredibly, there were even handwritten chemical formulae and (badly) hand-drawn spectra.

Molecular Materials forms Section C of *Molecular Crystals and Liquid Crystals Science and Technology*. All the members of the editorial board originate from Russia and former Soviet territories, which seems to contradict one of the stated aims of the journal, which is to attract "molecular materials scientists interested in . . .

international collaborations which are developing". Most of the manuscripts are from ex-Soviet laboratories. Are the papers being refereed exclusively from within ex-Soviet territories? If so, this is an unhealthy situation that is unlikely to attract manuscripts from around the world. We are all benefiting from the increased availability of reports of good-quality research in the former Soviet Union in our libraries, and this journal may assist the contributors in gaining international recognition for their work; but I am concerned that in the reference sections of many of the papers there is an overemphasis on citations from Russian journals that will be difficult to locate in the West. I am also aware of key references in Western journals that have been overlooked.

New materials, in all shapes, sizes and forms, are set to remain at the forefront of research for at least the next decade, so these new journals may survive, but at present they are not attracting any seminal publications or carving out any new niches. □

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German parochialism

Athene M. Donald

Acta Polymerica. Editors M. Ballauff, G. Hinrichsen, B. Philipp, A.-D. Schlüter, H.-W. Spiess and H. G. Zachmann. VCH. 6/yr. DM498, £183, \$360 (institutional); DM150, £57, \$125 (personal).

THIS journal is not strictly new. It has appeared under the same name for 40 years as a journal of the East German Academy of Sciences, but has recently been redirected with the aim of serving and reaching the wider community outside the former Eastern bloc. I do not believe the transformation is yet complete, and so the judgement passed here should perhaps still be regarded as provisional.

The primary authors of almost all the papers are, however, still German (although now from all parts) and the former Communist countries. The only papers from the United States that I have spotted, for instance, are from two members of the international advisory board. It is too early to tell whether the journal will succeed in overcoming this hurdle, but unless it does it is hard to see how it will gain credibility in its new role. Its coverage is not specific — "for researchers in all fields of polymer science" and "emphasizing an interdisciplinary approach, giving priority to the

physics and physical chemistry of polymers". It is not alone in hoping to do this, and the well established journal *Polymer* is an obvious rival in this area.

Publication time seems reasonable at around six months. But part of this relative rapidity is due to the fact that most papers are accepted as submitted, with a paltry minority indicating a date for receipt of a revised manuscript. The speed does not adequately reflect the quality of the papers, which largely seem to be worthy rather than cutting-edge. The production is adequate, although for photographs the matt quality of printing is less than desirable.

As it stands, I cannot see this journal cutting much of a swathe on the international scene. There are already highly respected journals on polymer science out there. There is a need for cross-fertilization between Eastern Europe and the West and it is possible that in time the journal will achieve this. Its price is in its favour, low enough for individuals to be tempted to subscribe in order to keep a watching brief on pan-German science, which for now is basically all they will be getting. □

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The future in sight

J. P. Oakley

Journal of Mathematical Imaging and Vision. Editor-in-chief Gerhard X. Ritter. Kluwer. 4/yr. DFL439.

THIS new journal will add to an already large array of titles in the field of image production, image processing and computer vision. Its stated editorial aim is to provide a forum for mathematical expositions in the field of imaging science. To this end, the editor has assembled an impressive editorial team of 30 well-known image specialists. Papers have been solicited from a vast technical area, ranging from biological vision to image processing and even electron optics.

The rationale behind the journal is that mathematicians are currently discouraged from working on imaging and vision because their output is seen as too 'mathematical' for engineering journals, yet too 'applied' for mathematics journals.

There does seem to be some historical evidence that a hard divide between mathematical and engineering journals has not served the imaging research community. An example is the theory of wavelets, which is now widely acknowledged to be an important mathematical resource as well as a tool with wide engineering applications. A large part of the motivation for the early work in this area came from image processing, and yet in 1988 Engrid Daubechies published a key paper entitled "Orthonormal basis of compact wavelets" in a mathematics journal, *Communications in Pure and Applied Mathematics*. Conversely, another important paper on a similar subject was published by Stephane Mallat in the engineering journal *Institute of Electrical and Electronic Engineers Transactions*, which is not widely read by Mallat's fellow mathematicians. The real communication between this group of workers seems to have been achieved using a mixture of circulated reports and personal letters. It could be argued that the theory of wavelets would have developed more quickly if there had been a high quality journal in a 'middle ground' between mathematics and engineering, particularly if the lead time to publication was short.

If *Journal of Mathematical Imaging and Vision* can provide encouragement to mathematicians to get their hands dirty with image-processing problems then this will be a good thing. The predicted market for image-related products in the next century is vast and there remains a host of technical problems to be solved, many of them of a deeply mathematical nature,

before the market is fully realized. It does seem a pity therefore that mathematicians are not better represented on the editorial board.

The first few issues contain many interesting papers of a highly competitive quality. The material does have a mathematical bias but no more than, say, the *IEEE Transactions on Pattern Analysis and Machine Intelligence* (the current top dog in this field). What is distinctive about the material in the new journal is that, in line with the editorial aim, a much wider range of topics is covered. The presentation is good. A compact size (roughly A5), coupled with a double-column format, a decent type size and top quality paper gives the impression of a polished and modern product. A reasonable personal subscription rate is available. □

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Accolades and brickbats

John Fox

Applied Intelligence: The International Journal of Artificial Intelligence, Neural Networks, and Complex Problem-Solving Technologies. Editor-in-chief Moonis Ali. Kluwer. 4/yr. DFL384.

The International Journal on Artificial Intelligence Tools. Editor N. G. Bourbakis. World Scientific. 4/yr. \$195 (institutional); \$100 (personal, and institutions from developing countries).

THE appearance of any new journal in the area of artificial intelligence (AI) must be viewed against a background of ten years of intense academic interest in the field and the concomitant rapid growth of publications.

Applied Intelligence is a primary research journal that aims to focus on the integration of AI techniques in constructing 'intelligent' computer systems. Each issue typically contains five papers of around 10,000 words each. *Artificial Intelligence Tools* is also a primary research journal but, as its name suggests, is more focused on tools and techniques than fundamental or applications research. It has about five papers per issue, though these are typically quite long, around 14,000 words each.

The preamble to *AI Tools* comments: "Artificial Intelligence has become an important and attractive research area offering many practical applications and solutions to scientists as well as other professionals. It has a rapidly increasing impact on our society and in the commer-

cial domain In response to these activities, a number of AI periodicals are being published worldwide. Most of them deal with the theory of AI and/or AI applications." Note the very positive assessment of AI's contribution so far and the sanguine reference to "a number of AI periodicals . . . published worldwide".

There is certainly a case for a journal of this kind. In recent years, AI has changed from a kind of empirical science to a discipline more like mathematics, and there are few outlets for practical papers about software methods. However, as 'tools' stray into 'techniques', the distinction between the kind of material published in *AI Tools* and that published in many other AI and computer science journals becomes rather blurred. I would judge that perhaps as many as 50 per cent of the articles in it could appear in any number of other AI journals. In addition, the range of topics covered seems very broad, and one wonders whether the journal has enough distinctiveness to establish itself on a long-term basis. Nevertheless, I found much of interest in the journal, and the quality of contributions is generally high.

The philosophy of *Applied Intelligence* is based on the claim that AI has come through two phases of development: the opening phase of research on basic questions, and then a phase of working on applications (notably expert systems). It is now moving, according to the editor, into a new phase with an "emphasis on the integration of multiple approaches in solving real-life, complex problems" in which real success will be achieved only by combining results and techniques from the earlier phases. The attitude to AI's current achievements is rather pessimistic by comparison with the upbeat assessment of the editor of *AI Tools*. This is typical of the whole field of AI, which attracts accolades and brickbats in about equal number; it would be nice to know the true position.

It is more surprising, given the apparently clear focus of the journal, that the four issues available for review have few papers on applications or on integration of methods. As with *AI Tools*, the journal does not yet appear to have carved out a distinctive style for itself. But the quality of the papers is high and the journal is attracting contributions from prominent workers. Perhaps this can in part be attributed to the impressive editorial board. Although membership of an editorial board is usually only symbolic (busy scientists have little time for editorial work), big names add credibility to a publication, and the editor has managed to sign up 70 of them.

I found little uniformity of style or structure in the presentation of the papers in either journal. This bothers me. It is a

general problem in AI that it has no accepted method or style of presenting research and results, in contrast with most experimental sciences. I do not ask for rigid rules and regulations, but AI needs to do a bit better. It is notoriously hard to judge 'good work' in the field and there is a lot of so-so work around — perhaps because there are so many journals to publish in. Scientific progress in any field is aided by agreed criteria for presenting, comparing and contrasting results. If AI improved its methodology, particularly in its applied research, it might encounter fewer brickbats.

In summary, both journals have faults, and I expect them to have a difficult passage. But they both have many virtues and I hope they will find their niche and their audience, despite the stiff competition. □

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Theoretical computing

John A. Campbell

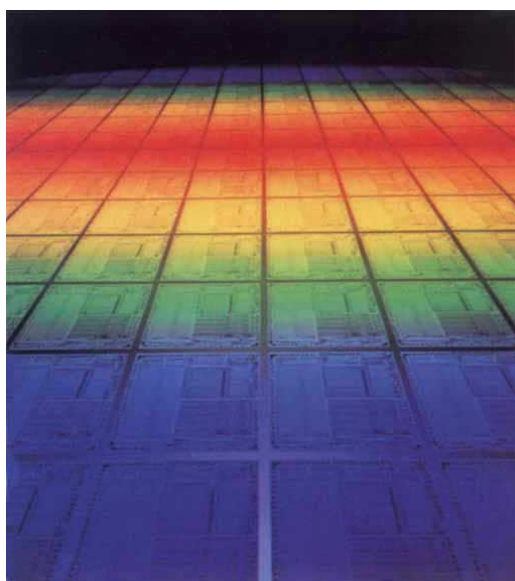
Journal of Logic and Computation. Editor-in-chief D. M. Gabbay. *Oxford University Press.* 6/yr. Europe £115, elsewhere \$215 (institutional); Europe £55, elsewhere \$99 (personal).

Applicable Algebra in Engineering, Communication and Computing. Managing Editor Jacques Calmet. *Springer.* 4/yr. DM258, \$161.

Parallel Processing Letters. Editor M. Cosnard. *World Scientific.* 4/yr. \$180 (institutional); \$90 (personal, and institutions from developing countries).

ON the evidence of these journals, theoretical computer science is experiencing a second wave of confidence. The first was marked by the launching of journals open to contributors from all corners of the subject and to people with new corners to suggest. Now computational theory is big enough, secure enough and demonstrably useful enough to justify journals that specialize in particular issues.

Journal of Logic and Computation admits logic-based research with relevance to any area of computer science. In practice, the bias of recent contributions has been towards logic programming and the specification of complex computing systems (for example for concurrent computations) in terms of logical statements about their desired properties. Other connections between logic and computation are possible, particularly in



David Parker/SPL

Parallel processing — macrophotograph of an array of rectangular transputers.

artificial intelligence, but have not been so much in evidence in the journal. An unusual feature is a regular polemical leading article. This may not have been intended to provoke letters to the editor (a category that the journal recognizes), but it deserves to. For specialists, the journal has both value and potential.

As logic tends to turn up within algebra as a topic in computer science degree courses, *Applicable Algebra* has an even wider coverage: anything algebraic that affects computing and information technology. In practice there is a traditional

division of interest that ensures that this journal concentrates on the kinds of algebra found in symbolic mathematical computation, coding theory or algebraic geometry. To give an idea of the coverage (which is again for specialists and researchers), the most commonly cited journal in bibliographies of its papers is *Mathematics of Computation*.

Parallel Processing Letters has two different flavours: it is intended as a 'letters' journal for rapid publication of fairly short papers, and it is (in principle, anyway) driven by the scientific area of computing that is named in its title. The advice to authors contains the intriguing message: "Experimental results are *also* welcome for publication if they contain an analysis corresponding to an abstract model of computation" (my italics). A possible

translation is: "Tell us why you think we theoreticians ought to pay any attention to your results".

Indeed, there is some evidence (well, just a little) in each journal that mathematicians rather than computer scientists are making the running. Subject to this culture warning, I shall be happy to ask my local research librarian to consider subscriptions to all three. □

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From the fundamental to the applied

Ian A. Walmsley

International Journal of Nonlinear Optical Physics. Editor-in-chief Iam-Choon Khoo. *World Scientific.* 4/yr. \$348 (institutional); \$170 (personal, and institutions from developing countries).

IN today's economic climate, it requires a degree of fortitude for a publisher to launch a highly specialized publication. But World Scientific has done an admirable job in laying the ground-work for this important new journal. The editor and members of the editorial board are all distinguished workers in nonlinear optics (including two of the founding fathers of the field, Professors N. Bloembergen and A. M. Prokhorov). Similarly, contributors to the first few issues comprise an international and well-known group of scientists and engineers.

Papers range from the fundamental to the applied, in roughly equal proportions, and the journal therefore competes directly with several more well-established

publications. As a newcomer, it is unlikely to be placed immediately among the journals read directly on publication; for now, it will find a niche as a repository for important and detailed information that needs to be in the literature but which will probably not revolutionize the field. But its status may rise as it becomes more established.

So far, issues have mainly contained lengthy research articles (10–20 pages each). "Rapid communications" (meaning, I presume, short articles) and lengthier review articles will also be published.

The production quality is high, although the layout and style of some of the figures occasionally interrupt the flow of the articles. Subscriptions are cheap and there are no page charges. In short, this is a useful addition to a comprehensive optics research library. □

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Mineral deposits

Mark Hannington

Exploration and Mining Geology. Editors J. F. Davies, H. L. Gibson and R. E. S. Whitehead. Pergamon. 4/yr. \$170, £110 (institutional); \$56, £36 (personal). (Membership rates on application to publisher.)

EXPLORATION AND MINING GEOLOGY is a quarterly publication of the Geological Society of the Canadian Institute of Mining, Metallurgy and Petroleum (CIM). The need for a publication devoted to the geology of mineral deposits was identified by the geology division of CIM in the early

in black shales, base metal deposits in eastern Canada and recent advances in diamond exploration. Future special issues will focus on specific mineral deposit types, important mineral districts and the mineral resources of different countries. While striving to provide information on the latest developments concerning mineral deposits in Canada, the new journal has also attracted papers on mining and exploration in other regions (such as China, Eastern Europe and Australia). Eight papers have come from outside Canada, and an editorial board with several international representatives will ensure future contributions from other countries. About two-thirds of the papers contain detailed field descriptions of the geological settings of deposits.



Tin men — mining for tin on Cerro Rico, "the rich mountain", in the Bolivian highlands, once a major source of silver. From *World Press Photo* 1993 (see page 569).

1980s, leading to the introduction of this journal in 1991. The journal is intended to provide the most up-to-date geological information available on important mineral deposit types and on the application of geochemistry, geophysics and geomathematics to the exploration and development of these resources.

In its first full year of publication, the journal has published more than 40 scientific papers of interest to the research geologist and to mining and exploration geologists alike. The papers are thoroughly reviewed and edited, and are of an excellent standard. Each of the first five issues contains eight or nine papers on topics as wide-ranging as the geology of individual deposits, new techniques in mineral exploration, exploration strategies and case histories, mining geology, process mineralogy, methods for calculating ore reserves and environmental geology applied to problems in mining. Three collections of papers arising mainly from conference proceedings have dealt with specific topics relevant to today's mineral industry, including mineralization

Nearly half of the papers are contributions from professional geologists active in the mineral industry. This is a significant departure from many journals dealing with ore deposits and an important opportunity for the journal: much of the critical information on exploration, mining and the geology of mineral deposits resides in the private sector. The willingness of today's mineral industry to open its doors reflects a growing appreciation of the importance of sharing this information in order to meet the rising demand for many metals and minerals in the face of declining reserves. This challenge will require improved geological models and exploration strategies for a variety of mineral deposits. It also demands greater communication with an international audience of mineral deposits geologists, geochemists and geophysicists. *Exploration and Mining Geology* is well-positioned to serve these needs. □

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Science in action

Michael B. Usher

Ecological Engineering: Journal of Ecotechnology. Editor-in-chief William J. Mitsch. Elsevier. 4/yr. DFL 361, \$195.

It is unusual to launch a new journal with a special issue devoted to a narrow theme within a broad subject area. A June 1991 workshop on "The role of created and natural wetlands in controlling nonpoint source pollution" provides the ten papers for the first two parts of *Ecological Engineering*. One therefore needs to explore the subject matter of the other eight papers, and three short communications, that complete volume 1 to gain an idea of what subjects the new journal covers.

The editor-in-chief, W. J. Mitsch, says: "Ecological engineering and ecotechnology combine basic and applied science for the restoration, design, and construction of aquatic and terrestrial ecosystems". The balance between aquatic and terrestrial environments is maintained, with papers on such diverse subjects as metal removal by wetland mesocosms, tertiary wastewater treatment using the root-zone method, land reclamation at a phosphate mine, and an expert system for establishing diverse wildflower grasslands. These glimpses of the subject matter demonstrate that papers will be relevant to many applied scientists.

The journal is attempting to gain an image that is not too academic. In the first four parts there is one letter to the editor (about large-scale transportation projects), one book review (on aquaria and how to build living ecosystems), one obituary (for Professor Ma Shijun) and announcements about future conferences. Such peripheral material is clearly important, and as the journal progresses the editors would be wise to focus on such information—it is likely to be of considerable benefit to applied scientists working on practical projects rather than to those in universities.

But, of course, the big question is "do we need such a journal?". Are there not already sufficient journals on terrestrial and aquatic conservation, environmental management or applied ecology? The new feature of this journal is the bringing together of the disciplines of engineering and ecology. It is true that very few of the first 18 papers actually achieve this merger. Perhaps that is to be expected in volume 1; the success of the journal will depend on how these subjects are synthesized in later parts, and how this new field will affect engineering work. □

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More than dinomania

Euan Nisbet

Earth: The Science of Our Planet. Editor Tom Yulsman. *Kalmbach Publishing Co., Box 1612, Waukesha, Wisconsin 53187, USA. 6/yr. USA \$19.95, elsewhere \$26.*

FEARSOME predators sometimes make good magazine covers. There is one predator so fearsome that its offspring happily amuse themselves by counting the teeth of *Tyrannosaurus rex*. Unfortunately, magazines with the most dangerous of all species on the cover tend to be dismal affairs; interesting to weightlifters



Tyrannosaurus rex rehabilitated.

perhaps, but of no significant content. But a good dinosaur, though a less dreadful animal, makes a finely tempting invitation to a magazine.

Earth is about dinosaurs. What else but an article on "Cretaceous Park"? Yet there is much more in this magazine than simply yet more dinos. The offering is a well-balanced meal of Earth science, prettily presented and easily digested. It is not supermarket checkout fodder, nor yet does it have the exquisitely dry gourmet delicacy of the review journals. It lies somewhere in the middle, the solid fare of the respectable bookshops. Pitched somewhat below the level of *Scientific American*, this is a magazine that would do excellently on the shelf of a high-school library, or in the waiting room of an up-market dentist.

The coverage in the first few issues is wide. Of course, the dinosaurs are there to draw the readers in. But the coverage of the big teeth is not disproportionate. There are stories about volcanoes, earthquakes and tsunamis, the Himalayas and climate, the mapping of the ocean floor

and the carbon cycle. The mix between topics is well representative of the diversity of Earth science, and the general impression is that an attempt is being made to provide a wide picture of the complexity of the planet.

A journal at this level, firmly popular, is not perhaps quite what *Nature* readers are interested in, but *Earth* is exactly the sort of magazine that science needs if good students are to be tempted back into the classroom. The stories are well researched, carefully balanced and cover all aspects of Earth science, not just Mesozoic monsters. There is a sense of reliability about the journalism, that facts have for the most part been checked, that good opinion has been sought. On this good basis, the writing is clear and

the illustrations attractive. The writers, who include some distinguished scientists, are clearly interested in their subject, and are not just hacks dashing off another piece of copy.

Earth is a magazine that deserves to succeed. If it is lucky it will ride the wave of *Jurassic Park* and firmly establish itself before dinomania wanes. In the longer term, such magazines can do much to interest students again in the pleasure and challenge of science. Science is seen as difficult and demanding, but the discipline and rigour are far less than that demanded by a football team. A magazine like this can show students that there is real enjoyment in learning how things work. It is not just students who will read this magazine. Perhaps the copies in the dentist's waiting room will make some small contribution to a wider general knowledge of the planet, and will help regain the interest of a society that has for the most part turned away from nature. □

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Practical policy and principles

Andrew Jordan

Environmental Values. Editor Alan Holland. *White Horse, 10 High Street, Knapwell, Cambridge CB3 8NR, UK. 4/yr. £64, \$110 (institutional); £32, \$60 (personal).*

ENVIRONMENTAL VALUES is "concerned with the basis and justification of environmental policy". It aims to bring together "contributions which relate to the present and future environment of humans and other species". It seeks to "clarify the relationship between practical policy issues and more fundamental underlying principles and assumptions".

These underlying tenets and belief systems are often dealt with either implicitly by decision makers and policy analysts or not at all. Nevertheless, it is important to be clear about what is valued about the environment and why, because questions of value often lie at the root of environmental conflicts and public-policy dilemmas. One of the most contentious issues is how one goes about measuring the 'value' of the environment. Another is how best to integrate the different dimensions of value — the economic, the bioethical, the inter-generational — into the practical process of decision making and policy formulation.

In his first editorial, Alan Holland underlines the fact that no single disciplinary perspective can claim to hold the key to environmental wisdom. Many would

concur with this judgement. Accordingly, he states that one aim of *Environmental Values* should be to initiate a "conversation across the disciplines".

Each issue includes an editorial and between three and six articles. There have also been review articles (for example, an informative summary of the concept of sustainability is included in issue four) and policy reviews. The editors have yet to make use of the 'special issue' format to cover particular topics or themes in greater detail. One issue includes a useful discussion section in which themes raised in an earlier article are debated by a reviewer and the original writer. There is a selection of book reviews (normally six to eight books per issue) and an announcements section in every issue.

In terms of readership, *Environmental Values* slots into a niche between exclusively policy-based journals such as *European Environment*, publications such as *Environmental Ethics* and more theory-biased disciplinary volumes such as the *Journal of Environmental Economics and Management*. *Environmental Values* will thrive if it plays to its undoubted strength, its interdisciplinary focus. But maintaining editorial direction for a cross-disciplinary journal with such a broad remit as *Environmental Values* is never going to be easy. Articles in the first volume have covered an interesting mélange of ethical, bioethical and economic concerns, but they have usually

been written from an economics and philosophy perspective. It would be beneficial to see more articles exploring the legal, ecological and political dimensions of environmental value.

The first volume is also uncomfortably dominated by UK and US writers. In developing countries the concept of environmental value can take on a very different form. Political and economic pressures may dictate that the environment is valued not so much for its aesthetic or amenity use, but because its very destruction helps to alleviate short-term poverty. More articles from authors in developing countries would certainly give the journal greater breadth and depth. But, these quibbles aside, *Environmental Values* is attractively produced and highly informative. It deserves to succeed. □

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Global concerns

Sara Naqvi

Down to Earth: Science and Environment Fortnightly. Editor Sunita Narain. *Society for Environmental Communications*, F-6 Kailash Colony, New Delhi 110 048, India. 26/yr. India Rs 1,000 (institutional), Rs 500 (schools), Rs 240 (personal); Bhutan, Nepal and Bangladesh \$80, \$45, \$40; Pakistan \$85, \$55, \$50; Sri Lanka and the Maldives \$90, \$55, \$50; elsewhere \$125, \$80, \$65 (airmail rates).

HAVING produced science programmes for the radio for more than a decade, I know that getting people to understand environmental issues is not an easy job. This magazine, however, covers these issues in such a comprehensible way that there will be few readers who fail to appreciate their global significance.

Down to Earth also publishes eyewitness accounts by people who are the real victims of those national and international policies that have caused these environmental problems. Creating a healthy balance between science, industry and the environment is an uphill struggle: the 1992 'Earth Summit' in Rio was a glaring example of this reality. The only way to overcome global problems is to make people aware of them, in particular scientists, industrialists, policy-makers and politicians.

The magazine's analyses are informative and provide many answers to common questions. Indian topics covered include child labour, women farm-workers,

deforestation and industrial pollution. Although many international agencies publish their own reports on these issues, reports at the grass-roots level are radically different in that they are truly down to earth. They also usually give insight into centuries-old problems from which millions of people are suffering yet which most Westerners find difficult to believe still exist.

A distinctive feature of this publication is that it does not foster any resentment towards science or industry; instead, it adopts a balanced approach and urges authorities to solve environmental problems by deeds rather than words. It will

Investigative zealousness

David Dickson

Probe. Editor David Zimmerman. *David Zimmerman Inc.*, 121 East 26th Street, New York, New York 10010, USA. 12/yr. USA \$53, Canada \$63, Europe \$70.

THE best newsletters — like the best journalism — are those that combine information with insight, and independence with imagination. An all-time classic was *I. F. Stone's Weekly*, a one-man operation produced by the veteran Washington journalist, which successfully punctured many of the myths put out by the Nixon and Johnson US presidential administrations about the conduct of the Vietnam War.

David Zimmerman, an experienced medical writer, explicitly seeks to emulate Stone's achievements with his publication *Probe*, a newsletter on "science, media, policy and health". Like Stone, Zimmerman uses his newsletter to convey a deeply personal perspective on the issues he is addressing, ranging from anti-cholesterol campaigns to scientific fraud. Indeed, it is his crusading desire to expose the personal and institutional links between the actors in a particular topic that gives the newsletter much of its appeal.

But there is a big difference. Stone's target was the hypocrisy of the establishment, and in particular the way its actions in Vietnam were masked in a deceptive language of peace and democracy. Zimmerman, by contrast, seeks to defend the values of the establishment — and in particular those of the scientific establishment — against its critics. His argument is that these critics are essentially anti-rationalist and anti-scientific. His newsletter aims to expose them as such.

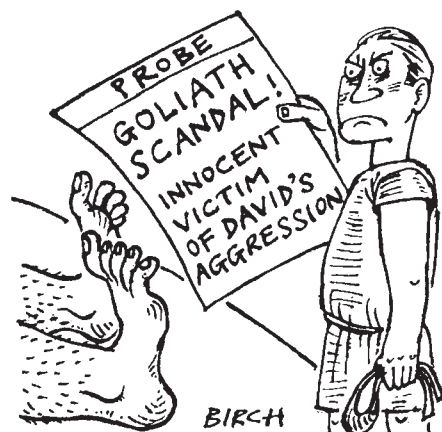
Take, for example, his coverage of the Baltimore/Imanishi-Kari affair at the Massachusetts Institute of Technology. Most of the media coverage has concentrated on Baltimore's treatment of Margot O'Toole, the postgraduate student whose

make a fine addition to all libraries. Readers will learn about the views of people in developing countries on many global problems and how these countries are coping with these dilemmas; they will also find detailed accounts of the attitudes of the developed world towards global policies and how much mess has been created by the developing countries' own policies and management. □

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questioning of the data published by Imanishi-Kari in a paper in *Cell* (co-authored by Baltimore) led to the paper being retracted by Baltimore.

Zimmerman takes Baltimore's side. For example, he emphasizes that the results reported in the *Cell* paper "had



been in large part confirmed and expanded by other researchers". And he draws attention to the minority view of a member of the congressional subcommittee that investigated the affair, who writes that anyone in his office who behaved as O'Toole had in copying Imanishi-Kari's notes would be "out of there in a flash".

Zimmerman's newsletter similarly defends geneticists against charges that investigations of the genetic precursors to violence have racist overtones. Indeed, it promotes the development and use of sophisticated drugs to curb violent behaviour. And it argues that the black community should welcome rather than criticize genetic studies, as these can be used to identify the precise African roots of black Americans.

The newsletter also goes in to bat for the medical establishment against promoters of "unconventional medicine", ranging from acupuncture (described as "mostly

useless") to homeopathy (described as "quackery"). For example, it makes much of the fact that the lead author of a study on the growing popularity of such practices published by the *New England Journal of Medicine* is a fellow of the Fetzer Institute, a body explicitly committed to integrating Eastern values into Western medicine.

There is much to be gained from *Probe's* investigative zeal. Partisanship (as Stone himself demonstrated) is no enemy of good journalism. The value of a free press lies in the belief that the imaginative exercise of a journalist's talents, whatever his personal convictions, is essential to an open democracy. And criticism of science can often, as Zimmerman points out, be based on inadequate information or misunderstanding.

But excessive partisanship can be self-defeating. And *Probe* frequently goes too far. It attacks the ideological partisanship of others (for example, those who criticize the profit-seeking of the drugs industry rather than that of health insurance companies). Yet it seeks to deny its own, claiming that all it is trying to do is to defend science as a "rational, self-correcting process of discovery".

This is a formula for lively writing. But, unlike Dan Greenberg's established *Science and Government Report*, *Probe* aims explicitly to head off critical debate on the dominant values of the scientific community. As such, it is not always a formula for good journalism. □

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Mixed bag

Carmen Pryce

Public Understanding of Science. Editor John Durant. IOP Publishing. 4/yr. £115, \$236 (institutional); USA and Canada \$81, Europe £31.50, elsewhere £37.75 (personal).

A STRAIGHTFORWARD title that says what it means and means what it says? I was expecting a readable scientific magazine full of colour pictures, enlightening graphics and articles from 'famous' living scientists who look like they've got it going on (Euro translation: they know that they are living in the 1990s and that the year 2000 is just around the corner). I was looking forward to something that would galvanize me, as a member of the public (which includes us all), into taking an active interest in science. I was wrong. So, as a broadcaster specializing in popular science, I read on. The review starts here.

Because one or two people think the Sun goes around the Earth, do we need "a reliable and valid multi-item scalar mea-

sure of scientific understanding... to assist in the analysis of the relationship between the science and the public" (J. Durant, G. Evans and G. Thomas)? And while 'the public' presumes that scientists make more mistakes than new discoveries, is it really worth studying the utterances of a Cumbrian sheep farmer and extrapolating the findings to everyone outside the world of science and technology?

Page upon page of scientific deliberation — which I assume corresponds to a lot of time and money — only to come out with such statements as: "The best communication process in the world cannot replace good government" (S. Bader and M. Shortland). Call me a party-pooper but this smacks of science for the sake of scientists.

This was my view of *PUS* until I got to the Chernobyl special issue. It was like reading an entirely different journal. Helene Knorre's "The star called Wormwood" was concise, informed, interesting and powerful (I didn't know that the English translation of 'Chernobyl' is 'Wormwood', which is also the name of a star, reference to which can be found in the Bible (Revelation 8: 10–11)). I pondered the idea of the article being smuggled out of the former Soviet Union, translated from hand-written Cyrillic into English and published only after the fall of Communism. The truth is probably more prosaic.

Although the Chernobyl issue did not keep up the pace, it didn't fall into the deadly uninteresting category. This is *PUS's* biggest problem; it's such a mixed bag, you never know what you're going to get. Vol. 2 No. 2 should be subtitled: 'Do not read more than the multi-lingual abstracts'. "Recepción y rechazo del conocimiento científico? elección, estilo y cultura familiar" — sounds fascinating.

On the other hand, the current issue could be subtitled: 'Everyone a winner'. It contains an article on screen portrayals of scientists, a study of science and technology reporting in British national newspapers (the *Guardian* and *The Times*), and a discussion about common sense (my favourite subject). I was happy and willing to read from cover to cover.

But the bottom line is that *PUS* seems generally to be scientifically written by socially conscious researchers for their brethren, scientists and technologists — with the appropriate jargon and sentiments to appeal. Any member of 'the public' who stumbles across *PUS* in the library is liable to have his or her beliefs and perceptions about the scientific fraternity well and truly confirmed. □

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Also submitted for review

The following is a list of journals received that were eligible for review but which for one reason or another are not covered in the preceding pages. The list does not include journals sent in that had not published enough titles to be considered.

Acta Diabetologica (Springer)
Animal Welfare (Universities Federation for Animal Welfare)
Aquatic Conservation (Wiley)
Epithelial Cell Biology (Springer)
Ethics and Behavior (Lawrence Erlbaum)
European Journal of Dermatology (John Libbey Eurotext)
Formal Methods in Systems Design (Kluwer)
Global Ecology and Biogeography Letters (Blackwell Scientific)
Industrial Crops and Products (Elsevier)
Integrated Ferroelectrics (Gordon & Breach)
International Journal of Aviation Psychology (Lawrence Erlbaum)
International Journal of Intelligent and Cooperative Information Systems (World Scientific)
International Journal of Methods in Psychiatric Research (Wiley)
International Journal of Modern Physics D: Gravitation, Astrophysics, Cosmology (World Scientific)
International Journal of Modern Physics E: Nuclear Physics (World Scientific)
International Journal of Oncology (National Hellenic Research Foundation)
Journal of Aquatic Ecosystem Health (Kluwer)
Journal of Biopharmaceutical Statistics (Dekker)
Journal of Consumer Psychology (Lawrence Erlbaum)
Journal of the Learning Sciences (Lawrence Erlbaum)
Journal of Narrative and Life History (Lawrence Erlbaum)
Journal of Nonparametric Statistics (Gordon & Breach)
Journal of Research on Adolescence (Lawrence Erlbaum)
Journal of Science Education and Technology (Plenum)
Journal of Systems Engineering (Springer)
Language Acquisition (Lawrence Erlbaum)
Machine Vibration (Springer)
Medicine, Exercise, Nutrition and Health (Blackwell Scientific)
Microbeam Analysis (VCH)
Nanobiology (Carfax)
Optimization Methods and Software (Gordon & Breach)
Oral Oncology (Pergamon)
Ornis Svecica (Swedish Ornithological Society)
Progress in Natural Science: Communication of State Key Laboratories of China (Science Press, Beijing, China)
Tobacco Control (BMJ Publishing Group)
Transgenic Research (Chapman & Hall)

The cause of Canavan's disease

Characterization of genetic defects involving myelin continues with the identification of the gene for Canavan's disease, a condition that results in the destruction of the brain's white matter.

ONCE an esoteric field confined to the neurological textbooks, the leukodystrophies continue to make the news. Not long after the release earlier this year of *Lorenzo's Oil*, a powerful film about a dietary treatment for the fatal demyelinating condition known as X-linked adrenoleukodystrophy (ALD), the gene for that disease was identified by positional cloning¹. In this month's *Nature Genetics*², Reuben Matalon, Rajinder Kaul and colleagues describe the cloning of the gene for a related autosomal recessive disorder called Canavan's disease, or spongy degeneration of the brain.

Canavan's disease, which was first described more than 60 years ago, primarily affects infants of Jewish origin. Many Jewish families with the disease have been traced back to founders in Lithuania and northern Poland. This region overlaps closely with the area in which Tay-Sachs disease, which is also common among Ashkenazi Jews, is thought to have arisen. Canavan's disease also seems to be unusually frequent in Saudi Arabia. Although its severity varies considerably — some patients die before the age of one, others live into their twenties — most patients die within a few years of birth. Mental retardation, muscle hypotonia (especially in the neck), macrocephaly and blindness are all common symptoms. At the histological level, the white matter of the brain shows severe demyelination and vacuolation, giving a customary spongy appearance.

For the past five years, there has been little doubt as to the identity of the gene for Canavan's disease. Matalon *et al.*³ showed that levels of *N*-acetylaspartic acid (NAA) in patients' urine were up to 200 times higher than normal and, by way of explanation, that the activity of the enzyme aspartoacylase, which processes NAA, was deficient in cultured fibroblasts from patients. Subsequent studies showed that levels of NAA, which is a normal component of brain neurons, are

greatly increased in the brain as well⁴. However, as seen in ALD, circumstantial biochemical clues can sometimes be misleading (the primary defect in ALD turned out not to be in a key enzyme, as had been widely suspected, but in a putative peroxisomal transporter¹). Indeed, formal proof of a genetic defect in aspartoacylase required Kaul *et al.*² to adopt a traditional approach: the laborious purification of bovine aspartoacylase, which then allowed them to isolate bovine complementary DNA clones, and eventually the human cDNA, which predicts a protein of 313 amino acids.

With the cDNA clones in hand, obtaining proof that the aspartoacylase gene is mutated in Canavan's disease came quickly. The first few patients examined were found to be homozygous for the same missense mutation — a glutamine to alanine substitution at residue 285. Taking advantage of a new restriction enzyme site created by the single nucleotide change, Kaul *et al.* screened a further 17 patients and found that 29 out of 34 (85 per cent) aspartoacylase alleles had the same missense mutation. Glutamine 285 is a conserved residue which the authors speculate forms part of an esterase-like catalytic site, and when mutated neatly explains the abolition of aspartoacylase hydrolytic activity.

The frequency of the common mutation in Canavan's disease points to a founder effect in the Jewish population, in agreement with earlier theories of the origin of the disease. Indeed, the new molecular data will help researchers to confirm whether recent estimates of the carrier frequency of Canavan's disease, perhaps as high as 1 in 30 among Ashkenazi Jews, are correct. Kaul *et al.* have recently identified two further aspartoacylase mutations, which will shed light on the possible relationship between specific mutations and the variable phenotypes that result. And just as Jewish couples contemplating marriage are routinely tested for Tay-Sachs disease, it will soon be possible — and advisable — for them to be screened for Canavan's disease once 95 per cent of the mutant alleles have been identified. On an equally practical level, a standard polymerase chain reaction assay will rapidly replace the former means of prenatal diagnosis, which involved attempts at measuring the very low aspartoacylase levels from cultured amniocytes.

Why should the failure to maintain normal levels of NAA in the brain produce the devastating effects on myelin, and why are they confined to the white matter? There are no good answers at present. Decreases in NAA are correlated with a number of disorders, including multiple sclerosis, neurodegenerative disorders and stroke, as monitored using nuclear magnetic resonance⁵, but these may be secondary manifestations. Interestingly, NAA levels are also increased in the grey matter in patients with Canavan's disease⁶, but this is spared any pathological damage.

Although much about the onset of the disease remains an enigma, the identification of the gene defect is yet another valuable piece in the myelin puzzle⁷. Several mutations in another form of X-linked leukodystrophy, Pelizaeus-Merzbacher disease, occur in a major myelin component called the proteolipid protein, and the enzyme defects in other leukodystrophies have been identified. In the peripheral nervous system, mutations in the peripheral myelin protein 22 gene and myelin protein zero give rise to the common hereditary neuropathies, Charcot-Marie-Tooth disease types 1A^{8,9} and 1B^{10,11}, respectively. Moreover, both genes have also undergone mutation in cases of a more severe condition, DeJérine-Sottas disease^{12,13}.

With the efficacy of Lorenzo's oil proving to be disappointing in clinical trials for a mild form of ALD¹⁴, hope for genuine therapies surely rests with the insights into myelin synthesis, structure and turnover that molecular genetics and the ensuing animal models are likely to provide.

Kevin Davies

Kevin Davies is Editor of Nature Genetics.

Also in this month's *Nature Genetics*: imprinting relaxation in Beckwith-Wiedemann syndrome; *de novo* mutations in Huntington's disease; screening for p53 mutations in yeast; mapping the locus for familial spastic paraplegia; and gene therapy advances in models for cystic fibrosis and muscular dystrophy.

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3. Matalon, R. *et al.* *Am. J. med. Genet.* **29**, 463-471 (1988).
4. Matalon, R. *et al.* *J. inher. metab. Dis.* **12**, 329-331 (1989).
5. Kauppinen, R. A., Williams, S. R., Busza, A. L. & van Bruggen, N. *Trends neurochem. Sci.* **16**, 88-95 (1993).
6. Kaul, R. *et al.* *J. Neurochem.* **56**, 129-135 (1991).
7. Aubourg, P. *Nature Genet.* **5**, 105-106 (1993).
8. Valentijn, L. J. *et al.* *Nature Genet.* **2**, 288-291 (1992).
9. Rao, B. B. *et al.* *New Engl. J. Med.* **329**, 96-101 (1993).
10. Hayasaka, K. *et al.* *Nature Genet.* **5**, 31-34 (1993).
11. Kulkens, T. *et al.* *Nature Genet.* **5**, 35-39 (1993).
12. Hayasaka, K. *et al.* *Nature Genet.* (in the press).
13. Roa, B. B. *et al.* *Nature Genet.* (in the press).
14. Aubourg, P. *et al.* *New Engl. J. Med.* **329**, 745-752 (1993).